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Unusual Chemical Constituents of *Lotus garcinii* (Fabaceae)

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The methanol soluble part of *Lotus garcinii,* belonging to the family Fabaceae, yielded three new interesting metabolites: garceine **(1)**, garoside **(2)** and garthiol **(3)**, which have never been detected from any natural source. In addition, isophytol, hexadecanoic acid, cholesterol, oleanolic acid, butulinic acid and lupeol were also obtained for the first time from *L. garcinii.* All the isolated metabolites were characterized by spectroscopic means.

Key Words: *Lotus garcinii*; Fabaceae; garceine; garoside; garthiol; characterization; spectroscopy.

Introduction

Leguminosae is the third largest family of flowering plants after Orchidaceae and Compositae, with approximately 650 genera and 18000 species. The family in general is characterized by the pod (legume) type of fruit. The members of this family are distributed throughout the world in almost all habitats ranging from wet lands to dry and cold deserts, from tropical forests to alpine habitats, and from sea level to 7000 m in the mighty Himalayan mountains[1]. They also encompass a wide variety of life forms as tiny herbs, vines, lianas and shrubs to gigantic trees in the forests. However, the greatest diversity of legumes is in the tropics and subtropics. Although about 18000 legume species are known in the world, only about 25 species are extensively utilized today. Yet it is only the legumes that have the potential to supply the vegetable protein that the ever growing population of the world needs. Keeping this in mind and also to judiciously exploit the legume resources of the world, an International Legume Database and Information Service (ILDIS) was initiated at the Royal Botanic Garden, Kew, U.K., to list and store information on all legumes of the world.

The family Leguminosae is divided into three sub-families[2]: Mimosidae, Caesalpinioideae and Faboideae. Due to the large size of these sub-families they are now treated as independent families and named Mimoaceae, Caesalpiniaceae and Fabaceae.

The genus *Lotus* belongs to the family Fabaceae, has 60-100 species, is most numerous around the Mediterranean and is represented in Pakistan by four species[3]. Their names are *L. garcinii*, *L. schimperi, L.*

makranicus and L. corniculatus. The chemical literature reveals that the various species of genus *Lotus* are rich in carbohydrates[4], triterpenoids and their saponins[5], steroids, coumarines, organic quaternary bases, tannins[6], flavonoids and their glycosides[7–9], proteins and nucleic acid, carotenoids[10] and nitro compounds[11].

Experimental

General Experimental: The IR spectra were taken on a JASCO A-302 spectrometer. Mass spectra were recorded on a Finnigan MAT-113 and 113 spectrometer coupled with the PDP 11/34 computer system. The NMR spectra were scanned on Bruker AM-400 and 300 spectrometers operating at 400 and 300 MHz for ^{1}H and 100 and 75 MHz for ^{13}C nuclei, respectively.

Collection and Identification: The plant material (fresh, 25 kg) was collected from Clifton (Karachi). It was identified by Prof. Khan Usmanghani Khan, Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, where the voucher specimen has been deposited (No.K-98-16) in the herbarium of the department.

Extraction and Isolation: After collection, the plant material was dried in the shade and then chopped. The dried and chopped material was then soaked in 18 liters of methanol for a period of two weeks. Methanol was removed at reduced temperature (28°C) to afford a gum (312 g). This gummy mass was diluted with distilled water and the organic material was recovered in ethyl acetate. The ethyl acetate soluble part was concentrated (67.7g) and chromatographed on a silica gel column. The mixtures of hexane, hexane-chloroform, chloroform, chloroform-methanol and finally, pure methanol were used as the mobile phase.

Similarly, the water layer was shaken with butanol and the butanol soluble part was concentrated into a thick syrupy liquid (612g). This was subjected to vacuum liquid chromatography (V.L.C.) using the various combinations of chloroform and methanol. The chloroform, chloroform - methanol and, finally, pure methanol were used as the mobile phase.

Garceine **(1)** was obtained with 25% chloroform in hexane as an impure sample from the column loaded with the ethyl acetate soluble part. This was then further cleaned by repeated chromatography. Elution with 20% chloroform in hexane yielded **1** as a white powder (14 mg).

M.P. : 60°C; **EI-MS:** *m/z* 366[M]$^{+}$, 267 [M-side chain]$^{+}$, 168[M- 2 × side chain]$^{+}$, 69[M-3 × side chain]$^{+}$; **FDMS:** *m/z* 366; **HR MS:** *m/z* 366.40111 (calcd. **m/z** 366.397380 for $C_{24}H_{50}N_2$); ^{1}**H NMR** ($CDCl_3$, 300 MHz): δ 0.86 (9H, t, J = 7.5 Hz, H-7′, 7″, 7‴), 1.24 (br.s, $(CH_2)_{16}$), 1.7 (1H, m, CH) and 4.20 (8H, m, H-1′, 1″, 3 and 5); ^{13}**C NMR** ($CDCl_3$, 75 MHz): δ 10.9 (CH_3), 14.0 (CH_3), 14.9 (CH_3), 29.0 (CH_2 of chains), 38.8 (C-4), 54.2 (C-1′ and C-1″), 63.1 (C-3 and C-5).

Compound **2** was eluted with 2% MeOH in $CHCl_3$ in crystalline form (16 mg) from the column loaded with the butanol soluble part of *L. garcinii.*

$[\alpha]_D$: + 37.9° (c 1.33, MeOH); **IR** (KBr) ν_{max}: 3410-3205 (br., OH) cm^{-1}; **EIMS**: *m/z* 336[M]$^{+}$, 321[M-Me]$^{+}$, 306[M-2xMe]$^{+}$, 273[M-Et$_3$C-]$^{+}$, 157[M-glc]$^{+}$; **FDMS**: *m/z* 336; **HRMS**: *m/z* 336.4013 calcd. *m/z* 336.39738 for $C_{16}H_{32}O_7$); ^{1}**H NMR** ($CDCl_3$+CD_3OD, 400 MHz): δ 1.05 (9H, J=7.5 Hz, 3×Me), 1.41 (3H, s), 1.42 (3H, s), 2.77 (6H, q, J=7.5 Hz, 3×CH_2), 3.12-3.79 (4H, CH of glucose) and 4.38 (1H, d, J=7.0 Hz, anomeric); ^{13}**C NMR** ($CDCl_3$+CD_3OD, 75 MHz): δ 10.8, 11.0 (Me), 13.1 (Me), 28.1 (Me), 28.7 (Me), 31.6 (3×CH_2), 62.9 (CH_2-glucose), 70.0, 73.2, 76.1, 76.4 (CH-glucose), 99.4 (anomeric) and 109.1 (O-C-O), 75.3 (C-Et$_3$).

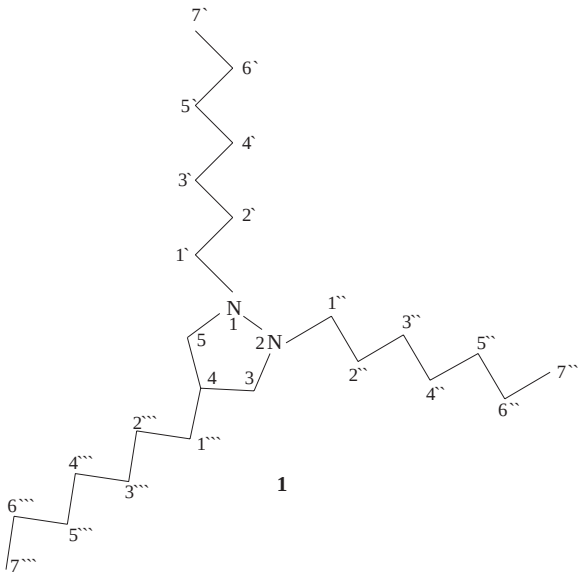
Just after the elution of **2**, compound **3**, was obtained, which was further purified by repeated CC using 5% methanol in chloroform to yield **3** as a foam (12 mg).

IR (KBr) ν_{max}: 3250 (br.), 2525 (SH) cm^{-1}; **EIMS**: *m/z* 196[M]CH^+, 163[M-SH]CH^+; **HRMS**: *m/z* 196.2511 (calcd. *m/z* 196.224 for $C_6H_{11}O_5S$); ^{1}**H NMR** (C_5D_5N, 400 MHz): δ 3.60-4.71 (6H, m); ^{13}**C NMR** (C_5D_5N, 100 MHz): δ 35.6 (C-1), 72.1, 72.9, 73.5, 74.0 and 74.5.

Results and Discussion

Compound **1** was eluted with 25% chloroform in hexane from the column which was loaded with the ethyl acetate soluble part of *L. garcinii*. The FD MS of **1** showed the molecular ion peak at *m/z* 366. Compound **1** on T.L.C. gave an orange spot by spraying with Dragendroff's reagent and the molecular weight of **1** is an even number, confirming that it contains the nitrogen atoms in even numbers. The peak matching of the molecular ion corresponded to the molecular formula $C_{24}H_{50}N_2$ (calcd. *m/z* 366.397380 observed *m/z* 366.40111). The molecular formula showed one degree of unsaturation, which may be a double bond or a carbonyl function or may contain a ring in the molecule. The absence of a double bond and carbonyl absorptions in the IR and NMR spectra confirmed the prresence of a cycle in **1**.

The proton spectrum of **1** exhibited the presence of three overlapped methyl triplets at δ 0.86 having the coupling constant 7.5 Hz due to the H-7′, H-7″ and H-7‴. The same spectrum also showed the signal at δ 1.24 as a broad singlet having the integration of 32 protons due to the three side chains attached to the N-1, N-2 and C-4. A downfield signal resonated at δ 4.20 as a multiplet due to the four methylenes attached to the nitrogen atoms through side chains (H-1′ and H-1″) and also in the ring (H-3 and H-5). The only methine (H-4) in the molecule is also a part of the ring appearing as a multiplet at δ 1.73 in the proton NMR spectrum with one proton integration. The DEPT spectrum of **1** displayed three methyl signals at δ 14.0, 10.9 and 14.9. The same spectrum also showed the downfield $(CH_2)_2$ resonated at δ 54.2 (C-1′ and C-1″) and 63.1 (C-3 and C-5). The only CH of the molecule appeared at δ 38.8 (C-4). The carbons of three side chains resonated at δ 29.0. The molecule does not contain any quaternary carbon.



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On the basis of the above discussed spectral data the structure of the compound is assigned as **1** and named garceine. The HMBC experiments did not help to determine the structure.

Compound **2** was purified from the butanolic part of *L. garcinii.* The EI mass spectrum of **2** showed a molecular ion peak at *m/z* 336. The formula corresponding to this peak was $C_{16}H_{32}O_7$, obtained from HRMS. The infrared spectrum of **2** showed the broad absorption at 3410-3205 cm^{-1} due to the hydroxyl function.

The proton NMR spectrum of **2** showed the presence of three methyl signals, which appeared at δ 1.05 as a common triplet having the coupling constant 7.5 Hz. At δ 2.77 a quartet (J=7.5 Hz) having the integration of six protons was found to be connected with the three methyl triplets. In this way, the three ethyl moieties were confirmed in the molecule. In addition to these three, two more methyl signals appeared at δ 1.41 and 1.42 as two close singlets. An anomeric signal resonated at δ 4.38 (J=7.0 Hz), indicating the presence of a sugar unit in the molecule.

The DEPT experiments of **2** confirmed the presence of five methyls appearing at δ 10.8, 11.0, 13.1, 28.1 and 28.7. The two downfield methyl signals at δ 28.1 and 28.7 were due to the methyls attached to the quaternary carbon appearing at δ 109.1. The downfield shift of this quaternary carbon (δ 109.1) was due to the attachment of two oxygen atoms to this. Another quaternary carbon, to which three ethyl units were attached, appeared at δ 75.3. The anomeric carbon resonated at δ 99.4. In the remaining carbons, chemical shifts of the sugar unit were exactly matched with the data of glucose[12].

The molecular formula showed that **2** contains at least one degree of unsaturation, which must be due to the sugar unit. The above discussion revealed that **2** contains three ethyl moities, a quaternary carbon connected to two oxygen atoms bearing the two methyls and a glucose unit. By joining all of these fragments it was concluded that the above discussed compound has the structure **2** and it was named garoside. The structure was further confirmed by the HMBC technique (Fig. 1).

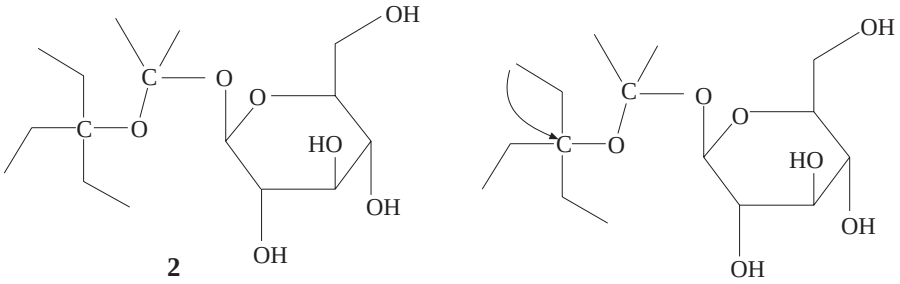

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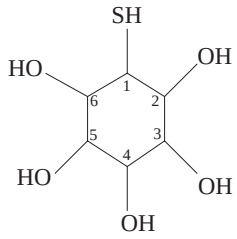
Figure 1. Selective HMBC connectivities

Compound **3** was also obtained from the butanol soluble part of the methanolic extract of *L. garcinii.* The EIMS of **3** showed a molecular ion-peak at *m/z* 196. The peak matching of *m/z* 196 showed the molecular formula $C_6H_{11}O_5S$. This formula indicated that the molecule contains at least one degree of unsaturation. As there is no indication of carbonyl or double bond functionalities in the NMR and IR spectra, it was concluded that the molecule contains a ring. However, the IR spectrum showed a medium intensity absorption at 2550 cm^{-1} due to the thiol (SH) function together with 3250(OH). The presence of a sulfur atom in the molecule was confirmed by element detection and it was found that **3** gave positive test results with lead acetate in the form of black precipitates, confirming the presence of sulfur in the molecule. A fragment at *m/z* 163 was due to loss of SH moiety.

The proton NMR of **3** was taken in pyridine because of the high polarity due to the polyhydroxyl nature. Due to the symmetry in the molecule, most of the carbinylic signals (CHOH) appeared in the range of δ 3.60-4.71 as a jungle of multiplets. Similarly, the broad band and DEPT spectra displayed only CH signals, whereas the quaternary carbons, methyls and methylenes were totally absent in the molecule. The CH signals appeared in the DEPT spectrum at δ 35.6, 72.1, 72.9, 73.5, 74.0 and 74.5 could not be numbered carbonwise. However, the most upfield CH signal at δ 35.6 was due to the direct attachment with the sulfur atom. The spectral analysis of **3** confirmed that **3** is a very simple molecule, having a cyclohexane ring containing a hydroxyl group at all the carbons except the one to which SH moiety is attached. The structure of compound **3** is assigned as 2,3,4,5,6-pentahydroxy cyclohexathiol and named garthiol.



Compounds **1-3** have neither been synthesized nor reported so far from any natural source and cannot be classified under any class of natural products. The bioactivity and stereochemistry of **1-3** could not be determined due to their minor amounts. The known constituents were confirmed through their spectroscopic data and comparison with the literature values. They include phytol[13], hexadecanoic acid, cholesterol[14], oleanolic acid[15], betulinic acid[15] and lupeol[16].

Acknowledgements

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