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ISOLATION AND STRUCTURAL ELUCIDATION OF A TRITERPENOID SAPONIN FROM GUAR, *CYAMOPSIS TETRAGONOLOBA*

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Key Word Index—*Cyamopsis tetragonoloba*; Leguminosae; guar meal; triterpenoid saponin; 3-epikatonic acid; 3-*O*-{[α-L-rhamnopyranosyl(1 → 2)]-[α-L-rhamnopyranosyl(1 → 4)]-β-D-glucopyranosyl(1 → 2)-β-D-glucuronopyranosyl(1 →)}-29-*O*-[α-D-glucopyranosyl(1 → 2)-D-glucopyranosyl(1 →)]-3β-hydroxyolean-12-en-29-oate.

Abstract—A new saponin has been isolated from the methanolic extract of the seed meal of *Cyamopsis tetragonoloba* and its structure elucidated as 3-*O*-{[α-L-rhamnopyranosyl(1 → 2)]-[α-L-rhamnopyranosyl(1 → 4)]-β-D-glucopyranosyl(1 → 2)-β-D-glucuronopyranosyl(1 →)}-29-*O*-[α-D-glucopyranosyl(1 → 2)-D-glucopyranosyl(1 →)]-3β-hydroxyolean-12-en-29-oate.

INTRODUCTION

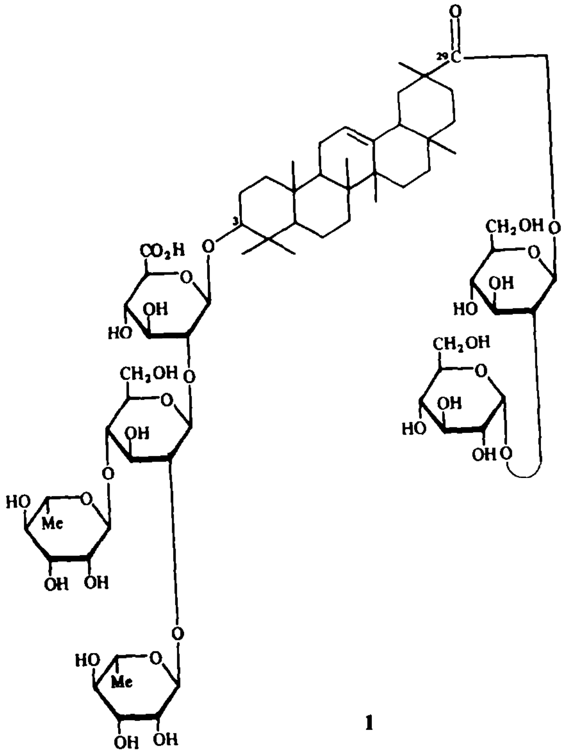
Guar, *Cyamopsis tetragonoloba* L. (syn. *C. psoraloides*), also known as the cluster bean, is indigenous to the Indian subcontinent where it is cultivated as a fodder and green manure crop. The immature green pods are cooked as a vegetable (like French beans) in India, Pakistan and Indonesia [1]. The plant is drought-resistant and was initially introduced to the drier areas of the U.S.A. as a fodder crop but it has since been grown commercially in the U.S.A., Australia and South Africa as a source of guar gum, a galactomannan used in the confectionery and cosmetic industries as a stabiliser, emulsifier and thickening agent. The meal remaining after extraction of the gum contains ~ 45% protein and it is a potentially valuable animal feed; however, its use in poultry, pig and cattle rations has been limited by its adverse effects on feed intake, growth and egg production [2, 3]. The physiological effects of guar meal have also been studied in rats [4, 5] and man [6].

This paper reports the isolation and structural identification of the major saponin present in guar meal.

RESULTS AND DISCUSSION

The methanol extract of the defatted guar meal was subjected to chromatography on silica gel, using chloroform, methanol, water mixtures, and afforded two major glycosidic compounds, **1** and **2**, both of which on alkaline hydrolysis yielded the de-esterified saponin analogues **3a** and **3b**, and saccharides **4a** and **4b**, respectively. On acid hydrolysis **1**, **2**, **3a** and **3b** all produced the aglycone 3-epikatonic acid (3β-hydroxyolean-12-en-29-oic acid), whose identity was confirmed by TLC and GC/MS comparison with an authentic sample [7].

Mass spectrometry, using fast atom bombardment (negative ion mode), produced spectra of **1**, **2**, **3a** and **3b** showing their molecular weights to be 1452, 1410, 1086 and 1086 respectively. The fragmentation patterns corresponded to the presence of six sugar units (namely one hexuronic, three hexoses and two deoxyhexoses) and one acetyl group in **1** while **2** gave the identical glycoside complement but without the acetyl group. The spectra of **3a** and **3b** were identical and corresponded to a tetrasaccharide moiety comprising one hexuronic acid, one hexose and two deoxyhexoses. Interpretation of these spectra suggested in both **1** and **2** the presence of a disaccharide (namely **4a** and **4b**) containing two hexoses attached to the C-29 carboxylic acid of the aglycone and a tetrasaccharide comprising two deoxyhexoses attached to a hexose–hexuronic acid moiety, with the uronic acid attached to C-3 of the aglycone. Standard sugar analysis



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(see Experimental) showed **3a** and **3b** to contain rhamnose and glucose (uronic acids not detected by this method), while **4a** and **4b** each contained glucose.

The 300 MHz ^{1}H NMR spectra of permethylated **3a** and **3b** were identical and exhibited two singlets at δ5.24 and 5.04 (the anomeric proton signals of two α-linked deoxyhexoses [8]) and two doublets at 4.72 ($J = 6.5$ Hz) and 4.30 ($J = 6.5$ Hz) (signals due to a β-linked hexose and a β-linked uronic acid, respectively). The 300 MHz ^{1}H NMR spectra of permethylated **4a** and **4b** were also identical and exhibited two doublets at δ4.59 and 4.90 ($J = 7.68$, $J = 3.11$ Hz) corresponding to a β-linked and an α-linked hexose, respectively. Integration of these two doublets gave a ratio of 1:3 showing that the terminal hexose attached to the C-29 ester was α-linked while the anomeric configuration of the 1,2-linked hexose was not determined.

Permethylated **3a** and **3b**, known to contain uronic acid residues, were reduced using $LiAlD_4$ (whereby the uronic acid moiety was converted to deuterated hexose). This stage was unnecessary for **4a** and **4b**, for which mass spectrometry by electron impact confirmed the absence of uronic acid (no ion at *m/z* 233).

Conversion of **3a**, **3b**, **4a** and **4b** to their partially permethylated alditol acetates and subsequent GC/MS analysis showed **3a** and **3b** to be identical, as were **4a** and **4b**. Individual sugars were identified according to a combination of their retention times and mass spectra [9]. Compounds **3a** and **3b** were found to contain two equivalent terminal rhamnose moieties (*m/z* 175, 162, 131, 118), a 1,2,4-linked glucose (*m/z* 233, 190) and a 1,2-linked glucuronic acid (*m/z* 234, 191, 190). Compounds **4a** and **4b** possessed a terminal glucose (*m/z* 205, 162, 161) and a 1,2-linked glucose (*m/z* 205, 190, 161). From the above evidence, **2** was characterized as 3-*O*{[α-L-rhamnopyranosyl(1 → 2)]-[α-L-rhamnopyranosyl(1 → 4)]-β-D-glucopyranosyl(1 → 2)-β-D-glucuronopyranosyl(1 →)}-29-*O*-[α-D-glucopyranosyl(1 → 2)-D-glucopyranosyl-(1 →)]-3β-hydroxyolean-12-en-29-oate, with **1** possessing an acetyl group attached to the C-3 tetrasaccharide moiety. From the data presented here is it is not possible to say whether compound **2** occurs naturally or is a degradation product of **1**.

EXPERIMENTAL

Isolation of saponins. Guar meal (500 g) was defatted by Soxhlet extraction with $CHCl_3$ (1.5 l., 16 hr). The air-dried defatted meal was then Soxhlet-extracted with MeOH (1.5 l., 30 hr). The MeOH extract was concentrated to a thick syrup then dissolved in a minimum vol. of H_2O and freeze-dried to yield 70 g crude extractant. This was dissolved in H_2O (30 ml) and applied to a column of reversed phase octasilane (C_8) bonded to silica gel (100 g) and eluted sequentially with H_2O (500 ml) and MeOH (500 ml). The MeOH fraction was evaporated to dryness (16 g) and an aliquot (1 g) dissolved in $CHCl_3$-MeOH-H_2O (10:9:1) and applied to a silica gel column (100 g). The column was eluted with $CHCl_3$-MeOH-H_2O (10:9:1) and the fractions collected were monitored by TLC [SiO_2-$(COOH)_2$, $CHCl_3$-MeOH-H_2O (65:35:10, lower layer) visualized with *p*-anisaldehyde–glacial acetic acid–97% H_2SO_4 (1:100:2) and heating] yielding a saponin-containing fraction (330 mg). Further fractionation of this material on a silica gel column (100 g) using $CHCl_3$-MeOH-H_2O (7:3:1, lower layer) resulted in the isolation of two major components (by TLC) which, on further purification on an RP SiO_2–C_8 column (10 g) using MeOH-H_2O (3:2), yielded 26 mg of **1** and 8 mg of **2**, respectively.

Structural analysis. Alkaline hydrolysis was carried out on an aliquot (5 mg) of each component in $NaBH_4$ (20 mM) in NaOH (0.1 N) at + 1–2° for 3 days. After neutralization (0.1 N HCl) the fragments arising from cleavage of the ester linkage were separated by fractionation on RP SiO_2–C_8 (3 g) eluted with H_2O (25 ml) yielding the ester-linked sugars (**4a** and **4b**) and then with MeOH to provide the residue (**3a** and **3b**)—the aglycone plus ether-linked tetrasaccharide. The fractions were either freeze-dried or evaporated to dryness, as necessary, and one-fifth was taken for standard sugar analysis. Compounds **3a**, **3b** and **4a**, **4b** were hydrolysed (HCO_2H, H_2SO_4), reduced ($NaBH_4$), acetylated (Ac_2O) and the resultant alditol acetates analysed by GLC.

The remaining portions of **3a**, **3b**, **4a** and **4b** were permethylated with dimsyl sodium (in DMSO) and MeI and submitted for ^{1}H NMR (300 MHz) examination. After reduction of uronic acids (**3a** and **3b** only) with $LiAlD_4$ the permethylated products were hydrolysed to the individual sugars (HCO_2H, H_2SO_4), reduced to alditols (BD_4^-), acetylated (Ac_2O) and the partially permethylated alditol acetates thus formed analysed by GLC and combined GC/MS.

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