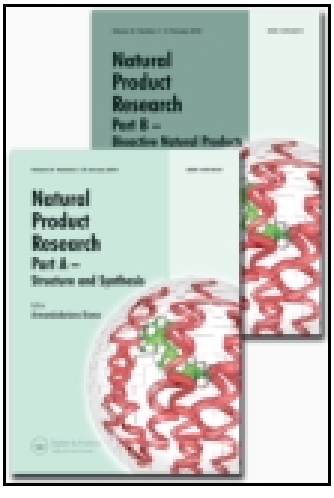

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SAPONINS OF *DIPLOCLISIA GLAUCESCENS*

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Abstract : The stem of *Diploclisia glaucescens* afforded *vibo*-quercitol and four new saponins, whose structures have been established. One saponin has shown acceptable molluscicidal activity.

Key Words : Menispermaceae, *Diploclisia glaucescens*, glucuronopyranosides, molluscicidal activity.

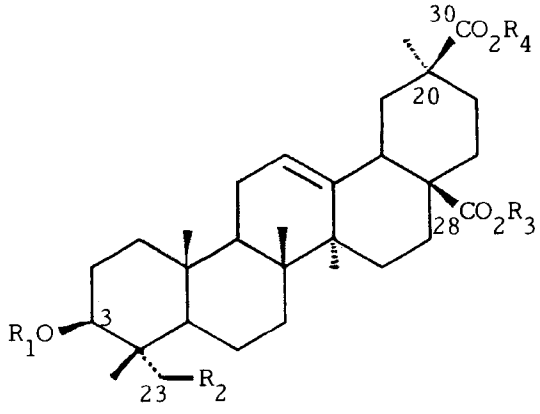
INTRODUCTION

Diploclisia glaucescens (Bl.) Diels of the family Menispermaceae is a creeper which grows in the mid-country regions of South India and Sri Lanka.[1] Two triterpenoid saponins have already been reported on separation of a methanol extract of the defatted mature stem of the plant over silica gel. Their structures have been established as 3-*O*-β-D-glucopyranosylphytolaccagenic acid[2] and 3,28-di-*O*-β-D-glucopyranosylphytolaccagenic acid (diploclisin).[3]

RESULTS AND DISCUSSION

After complete elution of diploclisin, the column was washed with methanol. Concentration of the methanol extract gave *vibo*-quercitol as colourless needles, m.p. 186°, $[\alpha]_D^{22}$ + 164° (MeOH). The identity of *vibo*-quercitol was established from its ^{1}H NMR and ^{13}C NMR data[4] as well as by comparison with an authentic sample. After separation of *vibo*-quercitol, the residual methanol extract was partitioned between *n*-butanol and water. The *n*-butanol extract showed strongly positive froth and hemolysis tests. It showed high spermicidal activity (100% immotility of spermatozoa of fresh human semen at 8mg/ml within 20 sec) and showed potential as a molluscicide (100% lethality to *Biomphalaria glabrata* snails at a minimum concentration of 50 ppm). The extract also showed mild anti-inflammatory activity (40% inhibition of carrageenan induced rat paw edema at a dose of 100mg/kg).

Repeated chromatography over silica gel of the *n*-butanol extract gave saponins I,II,III and IV in yields of 0.04%, 0.024%, 0.064% and 0.264% respectively. The purity of each compound was checked by HPLC.



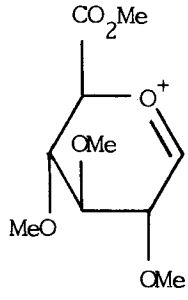
	R_1	R_2	R_3	R_4
I	glur	H	H	Me
Ia	glur	H	H	H
II	glur	OH	H	Me
IIa	glur	OH	H	H
III	glur	H	glc	Me
IV	glur	OH	glc	Me
V	H	H	H	Me
VI	H	OH	H	Me

glc = β-D-glucopyranosyl
glur= β-D-glucuronopyranosyl

Saponins I and II were isolated as microcrystalline needles, m.p. > 250°, $[\alpha]_D^{22}$ + 18.2° (MeOH) and m.p. 238°, $[\alpha]_D^{22}$ + 32.5° (MeOH) respectively. The IR spectrum of I showed absorptions at 3425, 1730, 1690 and 1080 cm^{-1}, and the IR spectrum of II showed absorptions at 3450, 1730, 1705 and 1080 cm^{-1} indicating the presence of hydroxyl, ester, carboxyl and glycosidic units in both compounds. The peaks at *m/z* 677.4 $[M+H]^+$ and 675.3 $[M-H]^-$ in the positive and negative ion FABMS of I gave evidence for the molecular formula $C_{37}H_{56}O_{11}$ of I. The peaks at *m/z* 715.4 $[M+Na]^+$ and 691.3 $[M-H]^-$ in the positive and negative ion FABMS of II indicated the molecular formula $C_{37}H_{56}O_{12}$ for II.

Hydrolysis of I with 4N HCl gave serjanic acid and D-glucuronic acid, whereas hydrolysis of II with the same reagent gave phytolaccagenic acid and D-glucuronic acid. Serjanic acid (V), $C_{31}H_{48}O_5$ and phytolaccagenic acid (VI), $C_{31}H_{48}O_6$ were identified by comparison with samples obtained from the same plant[2]. The peaks at *m/z* 483.4 $[C_{31}H_{48}O_5\text{-}OH]^+$ and 499.2 $[C_{31}H_{48}O_5\text{-}H]^-$ in the positive and negative ion FABMS of I gave further evidence for serjanic acid as the aglycone of I. Similarly, evidence for phytolaccagenic acid as the aglycone of II was provided by the appearance of a peak at *m/z* 515.3 $[C_{31}H_{48}O_6\text{-}H]^-$ in the negative ion FABMS of II.

Paper chromatography and TLC indicated that D-glucuronic acid was the sugar component in both I and II. Further confirmation was available from the EIMS of the pentamethyl and hexamethyl derivatives of I and II, obtained by methylating the saponins using a modified Hakomori procedure[5]. Appearance of an ion at *m/z* 233 in both spectra indicated a tetramethylglucuronic acid moiety.



Ion of *m/z* 233

The ^{13}C NMR spectra of I and II provided evidence for the position of attachment of D-glucuronic acid to the triterpenoid moiety in both compounds. Exclusion of C-28 as the position of attachment was evident from the C-28 signals at δ 181.08 and 181.34 in I and II respectively, indicating C-28 as free carboxyl in both compounds[6]. Attachment at C-3 was supported by the C-3 signals at δ 90.82 and 81.98 in I and II respectively.[6] The position of the C-23 signal at δ 64.78 in II excluded the possibility of attachment of the sugar moiety at C-23.[3] The configuration at each anomeric carbon atom in I and II was established as before[3] as β from the magnitude of the coupling constants (9 Hz) of the anomeric doublets in the ^1H NMR spectra of I and II. Thus I and II are 3-*O*-β-D-glucuronopyranosyl-serjanic acid and 3-*O*-β-D-glucuronopyranosylphytolaccagenic acid respectively.

The mass spectra of the pentamethyl and hexamethyl derivatives ($C_{42}H_{66}O_{11}$ and $C_{43}H_{68}O_{12}$) of I and II gave further evidence that the saponins are glucuronides. The CIMS (NH_3) of the pentamethyl derivative of I showed a strong $[M + NH_4]^+$ ion at *m/z* 764 and the FABMS of the hexamethyl derivative of II showed a strong $[M+H]^+$ ion at *m/z* 777. The EIMS showed $[M - HCO_2Me]^+$ ions at *m/z* 686 for the pentamethyl derivative of I and at *m/z* 716 for the hexamethyl derivative of II. Accurate mass measurement of the peak at *m/z* 686 was consistent with the molecular formula $C_{40}H_{62}O_9$.

The structures assigned to I and II were further confirmed by an analysis of all the carbon and proton resonances in their spectra as well as in the spectra of the products (Ia and IIa) of their alkaline hydrolysis. The structures of the latter are thus established as 3-*O*-β-D-glucuronopyranosylspergulagenic acid and 3-*O*-β-D-glucuronopyranosylesculentic acid respectively.

Hydrolysis of III with 4N HCl gave serjanic acid, D-glucuronic acid and D-glucose, whereas hydrolysis of IV gave phytolaccagenic acid, D-glucuronic acid and D-glucose. The presence of D-glucose in the hydrolysates of both III and IV was

established by reduction with $NaBH_4$ and acetylation to D-glucitol hexaacetate, identified by GC and GC-MS.[7] Further, permethylation of III and IV, acid hydrolysis, $NaBH_4$ reduction and acetylation gave 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol, identified by GC and GC-MS[7].

The presence of a D-glucose residue in III as well as IV was indicated in their FABMS. The peaks at *m/z* 877.4 $[M+K]^+$, 861 $[M+Na]^+$ and 837.5 $[M-H]^-$ in the positive and negative ion FABMS of III gave evidence for the molecular formula $C_{43}H_{66}O_{16}$ of III. The peaks at *m/z* 877.3 $[M+Na]^+$ and 853.2 $[M-H]^-$ in the positive and negative ion FABMS of IV indicated the molecular formula $C_{43}H_{66}O_{17}$ for IV.

The ^{13}C NMR spectra of III and IV gave evidence for the attachment of the D-glucopyranosyl moiety in each compound to C-28. Signals for C-28 and C-30 appeared at δ177.48 and 178.69 respectively for III and at δ177.44 and 178.65 respectively for IV. The C-28 attachment of the D-glucopyranosyl moiety was confirmed by alkaline hydrolysis of III and IV to Ia and IIa respectively. The configurations at both anomeric carbon atoms in each sugar moiety of III and IV were established as before[3] as β from the ^1H NMR spectra of both compounds. The structures assigned to III and IV were confirmed by an analysis of all the carbon and proton resonances in their spectra. Thus III and IV are 3-*O*-β-D-glucuronopyranosyl-28-*O*-β-D-glucopyranosylserjanic acid and 3-*O*-β-D-glucuronopyranosyl-28-*O*-β-D-glucopyranosylphytolaccagenic acid respectively.

The minimum concentration for 100% lethality in *Biomphalaria glabrata* snails was found to be 12, 30 and 50 ppm for I, II and III respectively. No activity was observed at 100 ppm for IV. Saponin III showed mild anti-inflammatory activity.

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