

YIAMOLOSIDE B, A FUNGISTATIC SAPONIN OF *PHYTOLACCA OCTANDRA**

M. MORENO and V. M. RODRÍGUEZ†

Instituto de Investigaciones Químico-Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Tzintzuntzan No. 173, Morelia, Mich., México

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All plants of the Phytolaccaceae are widely used in Oriental folk medicine for several diseases, such as edema and rheumatism [1]. Normally these plants are rich in saponins which have interesting biological activities (e.g. as anti-inflammatory agents and molluscicides [2, 3]). Among these plants, *Phytolacca octandra* has a well-proven use for some types of dermatitis. This aspect of its use is identical to that in ancient pre-Hispanic medicine and Mexican folk medicine [4–6] and has been confirmed by us in previous work [7].

This paper describes the isolation and structural determination of yiamoloside B, a new fungistatic saponin isolated from a biologically active mixture of triterpene saponins.

The biologically active material was partitionated from butanol and after butanol evaporation the residue showed six components on TLC analysis. The mixture was chromatographed on Si gel using CHCl_3 –MeOH– H_2O (30:10:1) as elution solvent. An active fraction ($\text{ED}_{50} = 3$ ppm *Tricophyton* sp.) was isolated in glassy form and was homogeneous on TLC. The R_f values were: CHCl_3 –MeOH– H_2O (30:10:1), 0.61; in EtOAc–EtOH– H_2O (65:35:10) lower phase, 0.71 and in EtOAc–EtOH– H_2O (15:5:4), 0.64. In the UV spectrum the compound showed a typical terminal absorption at 210 nm for a pentacyclic triterpene and gave a positive Liebermann–Burchard and Noller test.

After hydrolysis of yiamoloside B in 2 N H_2SO_4 –dioxane, galactose was obtained as the only sugar and was identified by TLC with BuOH–HOAc–Et₂O– H_2O (9:6:3:1), $R_f = 0.32$; EtOAc–pyridine– H_2O (8:2:1), $R_f = 0.20$; and CHCl_3 –MeOH–HOAc (75:30:15), $R_f = 0.39$. The organic layer of the hydrolysate gave serjanic acid as the genin (mmp, TLC, and MS) [8].

Yiamoloside B was acetylated in the usual manner to give an amorphous powder which showed the following signals in the ^1H NMR spectrum (60 MHz, CHCl_3): δ 0.70 (s, 2 × Me), 0.86 (s, 2 × Me), 1.15 (s, 2 × Me), 1.92, 2.03, 2.10, 2.13 and 2.20 (7 × COMe), 5.36 (2 H, axial acetate and vinylic hydrogen at C-12).

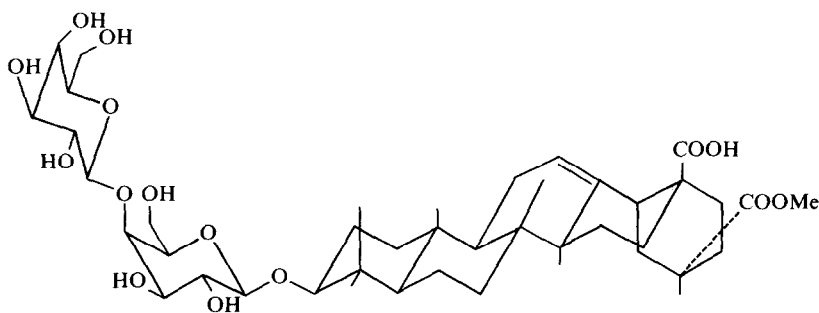
The presence of seven acetate signals in this product was indicative of not less than two hexoses in the molecule. Methylation of this product with ethereal diazomethane gave a methyl ester as needles from methanol, mp 209–212°: (C, 59.61; H, 7.20; O 33.10; calc. for $\text{C}_{58}\text{H}_{84}\text{O}_{22} \cdot 2 \text{H}_2\text{O}$: C, 59.66; H, 7.27; O, 32.93%). This compound showed a new signal for OMe in the ^1H NMR spectrum at δ 3.60 which revealed that the sugar moiety in the saponin was not at C-28 as an ester. ^1H NMR (100 MHz, CDCl_3): δ 0.71 (s, 26-Me), 0.73 (s, 24-Me), 0.90 (s, 25-Me and 23-Me), 1.12 and 1.14 (s, 27-Me and 30-Me) 1.98, 2.02, 2.04, 2.08, 2.12 and 2.16 (7 × COMe) 3.60 and 3.72 (2 × COOMe), 4.43 and 4.50 (both d, $J = 8$ Hz), 4.43 and 5.22 (m, 4 × CH) and 5.37 ($W_{1/2} = 4$ Hz, 2 × CH). The six methyl groups for serjanic acid were clear and corroborated the position of the sugar moiety in the aglycone. The hydrogens of the methyl groups at C-23 and C-24 shifted slightly but characteristic highfield shifts with respect to the methyl ester [9] and the singlets for both methyl groups were at 0.05 and 0.09 ppm respectively.

Two doublets for anomeric protons at δ 4.43 and 4.5 ($J = 8$ Hz) were indicative of the presence of two β -pyranosides; the anomeric protons for furanoses and α -pyranosides have a coupling constant of 4.5 Hz or less [10]. Signals were present in the spectrum for secondary acetate groups. One signal was for equatorial acetate groups and appeared between δ 4.43 and 5.22 and integrated for four hydrogens. The other signal was centred at 5.37 ($W_{1/2} = 4$ Hz) and integrated for two protons, one for an axial acetate and the other for the vinylic hydrogen at C-12. The existence of a proton at low field for an axial acetate indicates not less than one molecule of galactose [11] in the sugar moiety. The other natural hexose with an axial OH group, mannose, was rejected because of the coupling constant of the anomeric protons of our molecule ($J = 8$ Hz) in the case of rhamnose, with similar stereochemistry to mannose on C-2, the coupling constant of the anomeric protons is 1 Hz or less [12].

The detection of galactose as the only sugar in our molecule, and the detection in the ^1H NMR spectrum of only one equatorial proton for an acetate indicated a glycoside linkage of the type (1 → 4). Any other glycosidic bond would show two equatorial acetate protons. Hence

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† To whom correspondence should be addressed.



1

the structure of yiamolide B was established as serjanic acid 3-O- β -D-galactopyranosyl(1 \rightarrow 4)- β -D-galactopyranoside (1).

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