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PHYTOLACCOSIDE B: TRITERPENE GLUCOSIDE FROM *PHYTOLACCA AMERICANA*

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Key Word Index—*Phytolacca americana*; Phytolaccaceae; poke-weed; triterpenoid glucoside; phytolaccoside B.

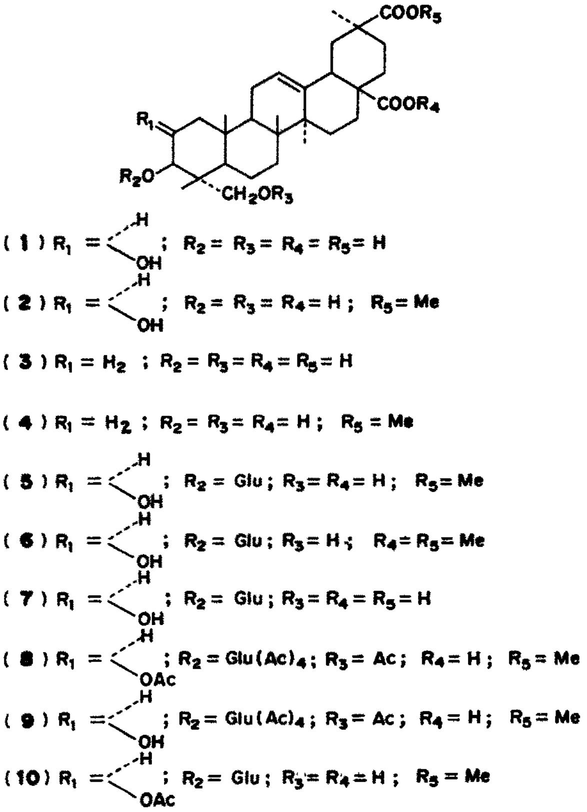
Abstract—The structure of phytolaccoside B, one of the major saponin components of the roots of *Phytolacca americana*, has been elucidated as 3β-D-glucopyranosyljaligonic acid 30-methyl ester.

The roots of *Phytolacca americana* are reputed in Korean medicine to alleviate rheumatism. They were found rich in saponins with strong anti-inflammatory activity [1]. According to earlier work [2,3] hydrolysis of the total saponin mixture yielded four sapogenins, jaligonic acid (**1**) [4] and its 30-methylester (**2**, phytolaccagenin) [5], and esculentic acid (**3**) [6] and its 30-methylester (**4**, phytolaccagenic acid) [7]. This paper describes the structural elucidation of phytolaccoside B, one of the major saponins isolated from the roots of this plant.

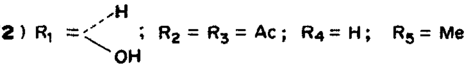
Phytolaccoside B (**5**), $C_{37}H_{58}O_{12}$, mp 215–218°, $[\alpha]_D^{25} + 75.8°$, on acid hydrolysis, gave jaligonic acid 30-methylester (**2**), mp 317–319°, as the genin, which was identified by direct comparison with an authentic sample (mmp, TLC, IR and MS). Glucose was identified in the hydrolysate from the saponin (**5**) by TLC. The yield of the genin in a quantitative hydrolysis experiment showed the presence of one sugar unit in the molecule (genin found 73%; calc. 76.66%).

Methylation of **5** with CH_2N_2 gave methylated product **6**, mp 182–184°, $[\alpha]_D^{25} + 71.6°$. Saponification of **5** with alkali afforded a new glucoside **7**, mp 234–239°, $[\alpha]_D^{25} + 77°$, which gave **6** by methylation with CH_2N_2. These results show that the sugar must be present in glucosidic linkage with one of the hydroxyl groups in ring A of the genin (not in ester linkage with the 28-carboxyl group).

In a quantitative periodate oxidation experiment 2 moles of the reagent were consumed. This indicates that the sugar is attached to the C-2 or C-3 position of the genin.



(**11**) $R_1 = O$; $R_2 = R_3 = R_4 = H$; $R_5 = Me$


(**12**) $R_1 = C(H)(OH)$; $R_2 = R_3 = Ac$; $R_4 = H$; $R_5 = Me$

(**13**) $R_1 = O$; $R_2 = Glu(Ac)_4$; $R_3 = Ac$; $R_4 = H$; $R_5 = Me$

Acetylation of **5** with acetic anhydride–pyridine on a steam bath afforded two compounds, a hexaacetate (**8**), mp 163–166°, $[\alpha]_D^{24} + 58.8°$, and a pentaacetate (**9**), mp 171–174°, $[\alpha]_D^{24} + 62.3°$, in a 7:3 ratio. When acetylated at 4°, the major product was **9**. Saponification of **9** with NH_3 gave the original saponin (**5**), however a monoacetate (**10**), mp 206–210°, $[\alpha]_D^{26} + 69.2°$, was formed from **8** under the same conditions. This result suggests that the more sterically hindered axial secondary alcohol group (i.e. 2β-OH) of the genin in the saponin (**5**) is free and therefore the sugar is linked to the 3β-OH group.

This suggestion was supported by Sarett oxidation of **9** followed by hydrolysis to yield 2-oxo-esculentic acid 30-methylester (**11**), mp 222–224°, λ_{max} 204 and 282 nm (log ϵ 3.67 and 2.63), which was identified by direct comparison with a sample prepared by oxidation of 3,23-diacetyl jaligonic acid 30-methylester (**12**), followed by hydrolysis.

The NMR spectra of **8**, **9** and **13**, in which the one proton doublets were observed at δ 4.52 (J 7 Hz), 4.50 (J 7.5 Hz) and 4.49 (J 6 Hz), respectively, indicated the β-configuration of the glucosidic linkage in **5**. This was also proved by Klyne's rule [8] (Molecular rotation difference between **2** and **5** = −84°, β-methyl-D-glucopyranoside = −62°). Thus the structure of phytolaccoside B was established as 3β-D-glucopyranosyljaligonic acid 30-methylester.

EXPERIMENTAL

Mp's are uncorrected. Optical rotations were measured in MeOH. IR spectra were recorded in KBr and UV spectra in EtOH. NMR spectra were taken in $CDCl_3$ using TMS as internal reference at 100 MHz.

Isolation of phytolaccoside B (**5**). The saponin mixture, obtained from a MeOH extract of *P. americana* roots as described previously [2], showed 10 spots on TLC (MeOH–$CHCl_3$–NH_4OH–H_2O 30:60:3:4). These were designated alphabetically in order of decreasing R_f values. The mixture was applied to a Si gel column and eluted with MeOH–$CHCl_3$ (3:97) to give a fraction which contained only two compounds, phytolaccoside A and B. Phytolaccoside B (**5**) was obtained by PLC, mp 215–218; $[\alpha]_D^{25} + 75.8°$ (MeOH; c 0.546); IR ν_{max}^{KBr} cm^{-1}: 3340 (OH), 2900 (Me, CH_2), 1725 and 1150 (methylester), 1700 (acid), 1000–1100 (glucoside); (Found; C, 63.89; H, 8.68. $C_{37}H_{58}O_{12}$ requires: C, 63.96; H, 8.41%). Lieberman–Burchard and Molish tests were positive.

Hydrolysis. The saponin (150 mg) was heated with 5% HCl–MeOH under reflux for 2 hr. The product was diluted with H_2O and extracted with Et_2O. The Et_2O extract was crystallized from MeOH to give needles of **2** (110 mg), mp 317–319°, which was identified by direct comparison with an authentic sample. The aq. part was examined by TLC (MeOH–$CHCl_3$–Me_2CO–NH_4OH 5:2:3:2) and it showed the presence of D-glucose only.

Methylation. The saponin (50 mg) in MeOH (20 ml) was treated with CH_2N_2 in the usual manner. The crude product was purified by PLC ($CHCl_3$–MeOH–NH_4OH–H_2O 20:4:1:3) and crystallized from MeOH to give needles of **6**, mp 182–184°; $[\alpha]_D^{25} + 71.6°$ (MeOH; c 0.725); IR ν_{max}^{KBr} cm^{-1}: 1725, 1150 (methylester); (Found: C, 63.95; H, 8.78. $C_{38}H_{60}O_{12}$ requires: C, 64.39; H, 8.53%).

Saponification. The saponin (100 mg) was heated under reflux in 20 ml of 3% NaOH–MeOH for 3 hr. The reaction mixture was acidified and extracted with BuOH. The BuOH extract was crystallized from MeOH to yield needles of **7**, mp 234–239°; $[\alpha]_D^{25} + 77°$ (MeOH; c 0.5); (Found: C, 63.44; H, 8.15. $C_{36}H_{56}O_{12}$ requires: C, 63.51; H, 8.29%). Methylation of **7** with CH_2N_2 gave **6**.

HIO_4 oxidation. A MeOH soln of the saponin (34.8 mg) was added to 0.01 M HIO_4 (20 ml), the volume made up to 35 ml with MeOH and the soln kept in the dark at 4°. Aliquots (7 ml each) were removed after 2, 4, 5, 28 and 35 hr. Estimation of the HIO_4 with 0.01 M Na_3AsO_3 in the usual way showed the consumption of 1.54, 1.85, 1.95, 1.98 and 1.98 mol of oxidant, respectively.

Acetylation. The saponin (100 mg) was heated with Ac_2O (3 ml) and C_5H_5N (2 ml) for 3 hr. Pouring onto ice gave solids which were chromatographed on Si gel and elution with MeOH–$CHCl_3$ (1:99) yielded **8** (65 mg) in the earlier fractions, mp 163–166°; $[\alpha]_D^{24} + 58.8°$ (MeOH; c 1.0); NMR; δ 0.74–1.16 (5 × Me), 2.05–2.13 (6 × MeCO), 3.72 (CH_3O), 4.52 (1H, d, J 7.0 Hz, anomeric proton); (Found: C, 62.21; H, 7.47. $C_{49}H_{70}O_{18}$ requires: C, 62.14; H, 7.45%). From the later fractions, **9** was obtained, mp 171–174°; $[\alpha]_D^{24} + 62.3°$ (MeOH; c 1.0); NMR: δ 0.71–1.24 (5 × Me) 2.04–2.09 (5 × MeCO), 3.69 (MeO), 4.50 (1H, d, J 7.5 Hz, anomeric proton); (Found: 62.31; H, 7.58. $C_{47}H_{68}O_{17}$ requires C, 62.37; H, 7.57%). On 10 hr treatment **9** was completely acetylated to give **8**. Partial acetylation of the saponin with Ac_2O–C_5H_5N at 4° overnight gave predominantly **9**.

Mild hydrolysis of saponin acetates. A sample (50 mg) of **8** in MeOH (7 ml) and NH_4OH (7 ml) was kept at 50° for 12 hr. The reaction mixture was concentrated under red pres and chromatographed on Si gel. Elution with MeOH–$CHCl_3$ (3:97) gave **10**, mp 206–210°, $[\alpha]_D^{26} + 69.2°$ (MeOH; c 0.5); (Found: C, 63.30; H, 8.22. $C_{39}H_{60}O_{13}$ requires: C, 63.57; H, 8.21%). On deacetylation under the same conditions, **9** yielded **5**.

Sarett oxidation of the pentaacetate (**9**). A sample (100 mg) of **9** in C_5H_5N (3 ml) was added to CrO_3 (100 mg) in C_5H_5N (2 ml) and left overnight at room temp. Since the oxidation product could not be separated from the unreacted compound, the mixture was acetylated to convert **9** into **8** and oxidised product (**13**) was separated by means of PLC ($CHCl_3$–MeOH–NH_4OH–H_2O 20:4:1:3), mp 155–157°; $[\alpha]_D^{25} + 39.5°$ (MeOH; c 0.66); NMR: δ 0.71–1.18 (5 × Me), 2.06–2.15 (5 × CH_3CO), 3.69 (CH_3O), 4.19 (1H, s, C-3), 4.49 (1H, d, J 6 Hz, anomeric proton); (Found: C, 62.49; H, 7.33. $C_{47}H_{66}O_{17}$ requires: C, 62.51; H, 7.37%). On acid hydrolysis **13** yielded the genin, mp 222–224°, which was identical with 2-oxo-esculentic acid 30-methylester (**11**) prepared as described below.

Synthesis of 2-oxo-esculentic acid 30-methylester (**11**). Partial acetylation of **2** with Ac_2O–C_5H_5N at 4° overnight gave 3,23-diacetyl jaligonic acid 30-methylester (**12**), mp 170–174°; $[\alpha]_D^{24} + 100°$ (MeOH, c 0.496); NMR: δ 0.74–1.33 (5 × Me), 2.10 (3H, s, MeCO), 2.18 (3H, s, MeCO), 3.72 (3H, s, MeO), 4.24 (1H, m, C-2), 4.49 (1H, d, J 3 Hz, C-3) and 5.37 (1H, m, C-12); (Found: C, 68.02; H, 8.45. $C_{35}H_{52}O_9$ requires: C, 68.16; 8.50%). A sample (70 mg) of **12** was oxidized with CrO_3–C_5H_5N in the usual way. The reaction product was deacetylated by refluxing in 3% HCl–MeOH, and chromatographed over Si gel. Elution with MeOH–$CHCl_3$ (2:98) gave **11** which was crystallized from Et_2O as flakes, mp 222–224°; UV λ_{max} nm (log ϵ) 204 (3.67), 282 (2.63); (Found: C, 70.27; H, 8.74. $C_{31}H_{46}O_7$ requires: C, 70.16; H, 8.74%).

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NIC-1-LACTONE, A MINOR STEROIDAL CONSTITUENT OF *NICANDRA PHYSALOIDES* (SOLANACEAE)

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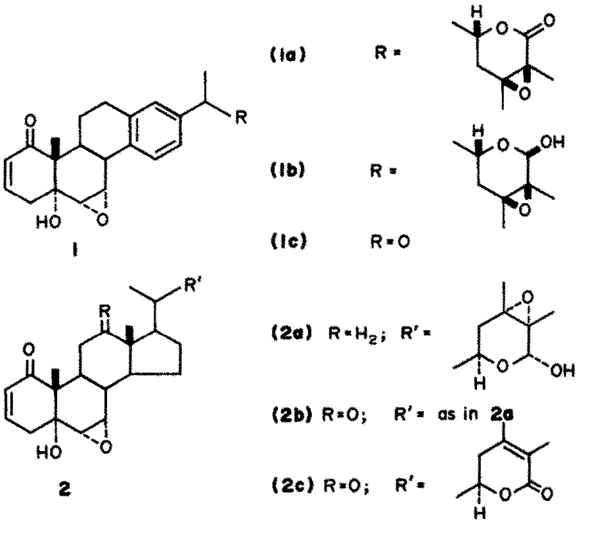
Key Word Index—*Nicandra physaloides*; Solanaceae; aromatic rang D; steroid; withanolides; Nic-1-lactone.

We wish to report on the isolation and characterization of Nic-1-lactone $C_{28}H_{32}O_6$, **1a**, mp 230–232°, a minor steroidal constituent of *Nicandra physaloides* (Solanaceae), the only plant known [1, 2] to contain steroids with an aromatic D ring. The compound was found to be identical with 6α,7α:24,25-diepoxy-5-hydroxy-17(13→18)abeo-5α-ergosta-2,13,15,17-tetraen-1-one-26,22-lactone, obtained [3] by oxidation with chromium trioxide of the lactolic C-26 hydroxyl group in Nic-1 (**1b**), the major steroidal component of *N. physaloides*.

The Nic constituents may be divided into two groups: (a) those with the usual steroid skeleton as encountered in Nic-3 (**2a**), Nic-7 (**2b**) [1] and withanicandrin (**2c**) [4]; (b) those with a rearranged skeleton, as found in Nic-1 (**1b**) [1, 2], obtained by expansion of ring D through inclusion of the C-13 methyl and subsequent aromatization. Most Nic constituents characterized so far possess an ergostane type side chain which has undergone oxidative processes leading to the closure of a six membered epoxy-lactol. In several compounds this side chain has gradually lost most of its carbon atoms, until a two carbon fragment remains as present in Nic-10 (**1c**) [1]. The usual unsaturated six membered ring lactone, characteristic for the majority of the withanolides [4], is found in only one compound withanicandrin (**2c**), occurring in this plant [4].

In our opinion, the epoxy-lactol system present in Nic-1-lactone (**1a**) is formed in Nature by oxidation of the lactolic hydroxyl group present in Nic-1 (**1b**) and not by epoxidation of the Δ^{24} double bond of an unsaturated lactone such as that present in the side chain of withanicandrin (**2c**). This assumption is based on the fact that all the solanaceous plants which produce withanolides, Nic-derivatives and physalins, possess enzymes which can catalyse the epoxidation of isolated double bonds, or double bonds in allylic systems, but which cannot epoxidize double bonds in $\alpha\beta$-unsaturated carbonyl systems.



EXPERIMENTAL

N. physaloides was raised in the nursery of the Volcani Center, Bet Dagon, Israel (introduction No. 20-707), from seeds received from Pretoria, South Africa (No. M-71-384). It is interesting to note that populations of plants raised from seeds received from India (Pondicherry) did not contain compound **1a**.

The isolation was done according to the described procedure [4]. The first fractions containing Nic-1, obtained by chromatography on a column of Si gel H, were contaminated by a product possessing a slightly higher R_f value. Separation was achieved by PLC on Si gel PF_{254}, 1 mm thickness, 40 cm length, developed with C_6H_6–EtOAc (1:4). The product possessing the higher R_f value (accounting for only 5% of the total amount of Nic-1) crystallised from Me_2CO–hexane, mp 230–232°, undepressed on admixture with 6α,7α:24,25-diepoxy-5-hydroxy-17(13→18)abeo-5α-ergosta-2,13,15,17-tetraen-1-one-26,22-lactone [3]. All physical constants and spectral data were superimposable.