Phytochemistry, 1974, Vol. 13, pp. 2887 to 2889. Pergamon Press. Printed in England.

# STEROIDS AND PENTACYCLIC TRITERPENOIDS FROM *PHYTOLACCA AMERICANA*

WON S. WOO
Natural Products Research Institute, Seoul National University, Seoul, Korea

(*Received* 2 *January*, 1974)

**Key Word Index**—*Phytolacca americana*, Phytolaccaceae, poke-root, pentacyclic triterpenoids, sterols, phytosterolins, palmityl sterol glucosides, jaligonic acid, phytolaccagenin, α-spinasterol, $\Delta^7$-stigmastenol

In the course of the study on the chemistry of vegetable drugs used in medicine from ancient times, the author had occasion to investigate triterpenoids and steroids in *Phytolacca americana* L in detail

*Triterpenoids.* Burke and Le Quesne,[1] from TLC result only, postulated the presence in the petrol. extract of roots of phytolaccagenin(30-monomethyljaligonate,[2] genin of phytolaccatoxin[3]), including oleanolic acid as free terpenoids, but complete identification was lacking, and no other triterpenoids were detected. From the ether extract of the roots, the author has isolated a considerable amount of jaligonic acid (**1**),[4] m.p. 319–320°, and a trace amount of phytolaccagenin (**2**), m.p. 317–319°, in the free state, which were identified by direct comparison with authentic samples[2,5] (m.m.p., TLC, IR and MS). Consideration of conditions of isolation as described in the Experimental rules out the possibility that **1** is an artefact arising from **2** or other saponins during extraction and separation process.

It was also found that a genin mixture from the total MeOH soluble saponins consisted of **2** (major component), **1**, and three unknown compounds, *A*, m.p. 229–230°, MW 484 ($M^+$), *B*, m.p. 309–311°, MW 516 ($M^+$), and *D*, all of which were not detected in the total $H_2O$ soluble saponins. MS of *A* and *B* showed typical retro-Diels–Alder fragmentation patterns expected for olean-12-enes and comparison with the spectrum of **2** upto *m/e* 300 shows a striking similarity in the ratios and an identity of position of the more important peaks, i.e *m/e* 292, 246, 232 and 187. The spectra, moreover, have a relatively intense peak each corresponding to the loss of COOH + H from molecular ion, but a peak corresponding to the loss of COOMe + H is hardly recognized in them.* MS fragmentation pattern of methyl ester of *A*, m.p 190°, MW 498 ($M^+$), is identical to those of dimethyl esters of **1**[2] and of spergulagenic acid [6] *A* showed a positive Zimmermann color test and could be obtained from **2** by treatment with acid under conditions as that of saponin hydrolysis Thus this compound would be an artefact formed from **2** during hydrolysis. *D* was very minor component which was difficult to purify, but its TLC behavior was identical with that of the product formed from *B* by alkali hydrolysis. Therefore, the structures of *A*, *B* and *D* are suggested to be 3-oxo-30-carbomethoxy-23-norolean-12-en-

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* MS of 30-monomethyljaligenate has a strong peak at *m/e* 486[M-(COOH + H)], whereas that of 28-monomethylester has a relatively intense peak at *m/e* 472 [M-(COOMe + H)] [5]

[1] BURKE, D E and LE QUESNE, P W (1971) *Phytochemistry* **10**, 3319

[2] WOO, W S (1973) *Lloydia* **36**, 326

[3] STOUT, G H, MALOFSKY, B M and STOUT, V F (1964) *J Am Chem Soc* **86**, 957

[4] WOO, W S (1971) *J Pharm Soc Korea* **15**, 99

[5] WOO, W S and KANG, S S (1973) *J Pharm Soc Korea* **17**, 152

[6] CHAKRABARTI, P, MUKHERJEE, D K, BARUA, A K and DAS, B C (1968) *Tetrahedron* **24**, 1107

28-oic acid, 3$\beta$,x-dihydroxy-30-carbomethoxy-olean-12-en-28-oic acid, and 3$\beta$,x-dihydroxy-olean-12-ene-28,30-dioic acid, respectively. For complete characterization of these structures, examination of fresh extract are in progress.

*Steroids.* Mixtures of $\alpha$-spinasterol (**3a**) and $\Delta^7$-stigmastenol (**3b**), $\alpha$-spinasteryl-D-glucoside (**4a**) and $\Delta^7$-stigmastenyl-D-glucoside (**4b**), and 6′-palmityl-$\alpha$-spinasteryl-D-glucoside (**5a**) and 6′-palmityl-$\Delta^7$-stigmastenyl-D-glucoside (**5b**) were obtained as inseparable crystals.

A mixture of **3a** + **b** possessed m.p. 166–168°, $[\alpha]_D^{13} = -4.8$ ($c = 0.83$ in $CHCl_3$), peaks at *m/e* 412 and 414 in the MS, and two peaks ($R_t$ 13·0 and 14·4 min) in the GC on 2% OV-17. NMR showed signals at $\delta$ 0.54 and 0·79 ppm for 18 and 19 methyl groups, respectively and IR has peaks at 3450 cm$^{-1}$ (OH), 965 cm$^{-1}$ (*trans*-disubstituted double bond), 840, 825 and 790 cm$^{-1}$ (tri-substituted double bond). GC–MS verified the presence of $\alpha$-spinasterol and $\Delta^7$-stigmastenol (20:1). Moreover, column chromatography over silica gel with $AgNO_3$ (15%) of the acetate, m.p. 178–179°, $[\alpha]_D^{16} = -1·9$ ($c = 1.3$ in $CHCl_3$) gave **3a**-acetate in pure state, m.p. 180–181°. The "phytosterol" ($C_{34}H_{56}O \cdot H_2O$, m.p. 166°, its acetate, m.p. 175–175.5°) of Jack and Rogers[7] from the berries of this plant would be identical with this mixture.

A mixture of **4a** + **b** showed m.p. 282–283°, $[\alpha]_D^{16} = -36.25$ ($c = 0·8$ in pyridine), absorptions in 1000–1100 cm$^{-1}$ region due to glycosidic bond in IR, two molecular ion peaks at *m/e* 574 and 576, peaks corresponding to mass of sterols at *m/e* 412 and 414, and peaks arising by cleavage of glucosidic bond at *m/e* 395 and 397 in MS[8,9] and gave glucose (TLC, GLC of TMS ether) and a mixture of **3a** + **b** (GLC, GC–MS) on hydrolysis with acid. The relatively large coupling constant for the anomeric proton ($J = 7$Hz) in NMR of the acetate, m.p. 174–175°, $[\alpha]_D^{15} = -18·5$ ($c = 0.65$ in $CHCl_3$), indicated $\beta$-anomer structure of this glucoside mixture.

A mixture of **5a** + **b**, m.p. 168–170°, showed in IR peaks at 1740 and 1165 cm$^{-1}$ (ester) and 717 cm$^{-1}$ (long $CH_2$ chain), besides peaks corresponding to $\alpha$-spinasteryl glucoside. MS showed two molecular ion peaks at *m/e* 812 and 814 and fragmentation peaks at *m/e* 574 and 576, corresponding to sterol glucosides, 412 and 414, sterols, 401, pyronium ion of palmityl glucose moiety, and 395 and 397, sterol parts. On hydrolysis with alkali, it gave palmitic acid (GLC of methyl ester) and a substance, whose properties were identical to those of the mixture of **4a** + **b**. The final evidence for the structure was provided by the formation of HCOOH by $HIO_4$ oxidation. To the best of our knowledge the isolation of **5a** and **5b** from a natural source seems not to have been reported earlier.

## EXPERIMENTAL

*Plant.* Raised from seeds collected in the wild in the vicinity of Seoul and grown in experimental garden. The voucher specimen is deposited in the Herbarium of this Institute.

*Extraction of free terpenoids.* The homogenized fresh roots were extracted with $Et_2O$. The $Et_2O$ layer was partitioned with saturated $NaHCO_3$ and then with 5% NaOH. Ppts formed by adding *d*-HCl in each of the alkaline soln were chromatographed over $SiO_2$ (eluent: $CHCl_3$–MeOH–7% HOAc = 5:1:1, lower) separately, to give **1** and **2**, respectively.

*Extraction and hydrolysis of saponins.* The methanolic extractive of the dried roots was separated into three fractions, $H_2O$ soluble, MeOH readily soluble and less soluble. The $H_2O$ soluble fraction was extracted with BuOH. After removal of solvent, the $H_2O$ soluble saponins were precipitated by addition of $Et_2O$ into MeOH soln of the residue. MeOH readily soluble fraction was continuously extracted with $Et_2O$ until free terpenoids were removed and saponins were precipitated by addition of $Et_2O$ into MeOH soln of $Et_2O$-insoluble material.

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[7] JACK, L. D. and ROGERS, C. H. (1942) *J. Am. Pharm. Assoc.* **31**, 81.

[8] NIGAM, S. K., MISRA, G. and MITRA, C. R. (1973) *Planta Med.* **23**, 145.

[9] WOO, W. S. and KANG, S. S. (1973) *J. Pharm. Soc. Korea* **17**, 161.

Hydrolysis of saponins in a mixture of dioxane–HCl (6 1) and column chromatography on $SiO_2$ as before gave five components which have been designated as Genin A–E in order of increasing polarity on TLC Genin C and E were identical with **2** and **1**, respectively, by direct comparison Genin A, *m/e*· 484 (M, 1 4), 438[M-(COOH + H), 13 2], 424[M-(COOMe + H), 3 4], 378[M-(COOH + H)-(COOMe + H), 3 9], 292(RDA fragment *a*, 19), 246[*a*-(COOH + H), 56 6], 233[*a*-(C-COOH + 2H), 20 2], 232[*a*-(COOMe + H), 19], 219(12 3), 215(12 3), 191(RDA fragment *b*, 9 5), 187[*a*-(COOH + COOMe + H), 100], 186[*a*-(COOH + H)-(COOMe + H), 53 2], and 173[232-(C-COOH + 2H), 32 5] Methyl-Genin A, *m/e* 498(M, 5 1), 438[M-(COOMe + H) 26 9] 378[M-2(COOMe + H), 24 3], 306(RDA fragment *a*, 38 4), 246[*a*-(COOMe + H), 44 8], 233[*a*-(C-COOMe + 2H), 41], 215(17 9), 191(RDA fragment *b*, 10 2), 187[*a*-(2COOMe + H), 100], 186[*a*-2(COOMe + H), 66 6] and 173[246-(C-COOMe + 2H), 51 2] Genin B, *m/e* 516(M, 1), 498(M-$H_2O$, 2 1), 480(M-$2H_2O$, 2 6), 470[M-(COOH + H), 8 3], 456[M-(COOMe + H), 2 3], 292(86 2), 246(100), 233(13 2), 232(22 2), 223(RDA fragment *b*, 8 3), 219(11 8), 215(18 1), 187(73 7), 186(44 5) and 173(25 7)

*Separation of steroids* The MeOH less soluble fraction was extracted with $CHCl_3$ Insoluble residue was crystallized from $CHCl_3$–MeOH(1 1) to give **4a** + **b** The residue from the $CHCl_3$ extract was chromatographed over $SiO_2$ Elution with $CHCl_3$ yielded **3a** + **b** and elution with $CHCl_3$–MeOH–$NH_4OH$–$H_2O$(20 4 1 3, lower) gave **5a** + **b**

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Phytochemistry 1974 Vol 13, pp 2889 to 2890 Pergamon Press Printed in England

# AMIDES OF *PIPER CHABA*

AMARENDRA PATRA and ARUNDHATI GHOSH

Department of Chemistry, University College of Science, Calcutta—9, India

(*Received* 8 *April* 1974)

**Key Word Index**– *Piper chaba*, Piperaceae, alkamides, piperine, sylvatine, piperlonguminine

*Plant. Piper chaba* Hunter (Piperaceae) is a climbing glabrous (rather fleshy) creeper, cultivated in various parts of India and Malaya Islands[1] The roots and fruits find numerous applications in medicine, particularly useful in asthma, bronchitis, fever, pain in abdomen, as stimulant and in haemorrhoidal affections.[1] Earlier work[2] on the stems of this plant resulted in the isolation of piperine (**1**). The present investigation on the roots of this plant has resulted in the isolation of two other related alkamides (**2**, **3**) and sitosterol.

The air dried milled roots of *P. chaba* (6·5 kg) were soxhletted with petrol. (60–80°) for 40 hr. Removal of the solvent gave a semi-crystalline mass which was treated with $Et_2O$ and left at 5° overnight. The crystalline residue separated was filtered off. The residue upon chromatography over silica gel afforded a light yellow compound crystallizing from $C_6H_6$–petrol. in needles (19 g) m.p. 129°, subsequently identified as piperine (**1**). The residue from the mother liquor upon chromatography over silica gel afforded sitosterol (m m p , IR and $R_f$ values). $C_6H_6$ eluted first sylvatine[3] (**2**), m.p 112°, crystallizing in flakes (320 mg) from $C_6H_6$–petrol. and later piperine (~4 g). The residue from $C_6H_6$–$CHCl_3$

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[2] BOSE, P K (1935) *Sci & Cult* **1**, 111

[3] BANERJI, A and GHOSH, P C (1973) *Tetrahedron* **29**, 977