β-Sitosterol. C₂₉H₅₀O (found: C, 83.98; H, 12.15; required: C, 84.05; H, 12.07, IR, NMR, m/e 414 (M⁺). [a]_D, m.p., mixed m.p., of sterol and its acetate). From benzene-CHCl₃ fractions and crystallizations with acetone. Alcoholic extract was extracted with Et₂O. Extract on repeated crystallizations with EtOH gave ursolic acid. C₃₀H₄₈O₃ (found: C, 78.80; H, 10.58; required: C, 78.94, H, 10.52, m.p., mixed m.p., [a]_D, m/e 456 (M⁺); co-TLC, m.p., methyl ester and formate).

Glucose, fructose and sucrose have been identified in the aq. solution by paper chromatography.

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PHYTOLACCACEAE

3-ACETYLOLEANOLIC ACID FROM PHYTOLACCA AMERICANA SEEDS*

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Abstract—3-Acetyloleanolic acid has been obtained from the seeds of *Phytolacca americana* L. It has not been detected in other parts of the plant. Oleanolic acid has been detected in the roots.

INTRODUCTION

THE ROOTS of *Phytolacca americana*, abundant in the eastern United States, owe their toxicity to phytolaccatoxin, a disaccharide (xylose, glucose) of phytolaccagenin (I). Since phytolaccagenin probably arises biogenetically from oleanolic acid (II), we have searched for related compounds in the plant which might reflect aspects of this conversion.

RESULTS

Light petroleum extraction of the seeds of *P. americana* gave oily material from which 3-acetyloleanolic acid (III) was obtained in 0·17 per cent yield. The seeds were further extracted with methanol. No other triterpenoids were detected in either extract.

¹ G. H. STOUT, B. M. MALOFSKY and V. F. STOUT, J. Am. Chem. Soc. 86, 957 (1964).

An extraction of the roots yielded, as free triterpenoids, traces of oleanolic acid (II) and phytolaccagenin (I), detected chromatographically. No other triterpenes were detected. It is possible that the phytolaccagenin arises by hydrolysis of phytolaccatoxin during preparation of the plant material. The juice of mature berries of *P. americana* foams on shaking with water, but acid hydrolysis of freeze-dried juice gave no detectable triterpenoids.

These results suggest that phytolaccagenin (I) arises in vivo by hydroxylation of oleanolic acid (II); the occurrence of (III) as the only other triterpenoid detected suggests that acetylation of the C-3-hydroxyl group may block the further hydroxylation leading to phytolaccagenin.

EXPERIMENTAL

Extraction of P. americana seeds. The finely-ground seeds (650 g) were extracted (Soxhlet) with petroleum ether (b.p. $30-60^{\circ}$) for 30 hr. Concentration of the extract gave a yellow oil, which during 24 hr deposited fine needles (1·1 g) m.p. 290-300°. Recrystallization once from EtOAc-EtOH gave pure 3-acetyloleanolic acid, m.p. 290-300° identified by IR, NMR, and mass spectra, and preparation of oleanolic acid (hydrolysis), methyl oleanolate (hydrolysis and methylation with CH_2N_2), and methyl 3-acetyloleanolate (methylation with CH_2N_2). The physical constants of these three derivatives were in exact accord with the literature.

Extraction of P. americana roots. The dried, ground root (70 g) was extracted with petroleum ether (b.p. 30-60°) (Soxhlet) for 72 hr. The extract was concentrated and analysed by TLC, using Eastman Chromagram silica gel plates with toluene-EtOAc-HOAc (12.4:0·5)⁴ and SbCl₃ in CHCl₃ as spray reagent. Phytolaccagenin and oleanolic acid were identified by co-chromatography against authentic samples. No other triterpenoids were detected by this method.

Extraction of P. americana berry juice. The freeze-dried juice (100 g) was heated under reflux with 10% HCl in MeOH-H₂O (1:4) (500 ml) for 10 hr. The mixture was partly neutralized with NaHCO₃ and extracted with ether. The ether layer was decolorized (Norit), dried (MgSO₄) and concentrated to small volume. This was subjected to TLC as above. No triterpenoids were detected.

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- ² C. DJERASSI, H. BUDZIKIEWICZ and J. M. WILSON, Tetrahedron Letters No. 7, 263 (1962).
- ³ Les Triterpenoides en Physiologie Vegetale et Animale (edited by P. Boiteau, B. Pasich and A. Rakoto Ratsimamanga), p. 139, Gauthier-Villars, Paris (1964).
- ⁴ M. H. A. ELGAMAL and M. B. E. FAYAZ, Z. Analyt. Chem 211, 190 (1965).

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RANUNCULACEAE

ALKALOIDS OF ACONITUM VIOLACEUM

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Aconitum violaceum Jacq. is available in Azad Kashmir. The roots of the plant are reported to be poisonous according to some, non-poisonous according to others and are eaten by the hillmen of Kunwar as a pleasant tonic. Although, other Aconitum species, such as

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- ¹ R. N. CHOPRA, S. L. NAYAR and I. C. CHOPRA, Glossary of Indian Medicinal Plants, p. 5, Council of Scientific and Industrial Research, New Delhi (1956).