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°) 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,[2] 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.[3]

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 $SbCl_3$ in $CHCl_3$ 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–H_2O (1:4) (500 ml) for 10 hr. The mixture was partly neutralized with $NaHCO_3$ and extracted with ether. The ether layer was decolorized (Norit), dried ($MgSO_4$) and concentrated to small volume. This was subjected to TLC as above. No triterpenoids were detected.

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RANUNCULACEAE

ALKALOIDS OF *ACONITUM VIOLACEUM*

G. A. MIANA,* M. IKRAM, M. ISRAR KHAN and F. SULTANA
P.C.S.I.R. Laboratories, Peshawar, P.O. Peshawar University, Pakistan

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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.[1] Although, other *Aconitum* species, such as

* Present address: Institute of Chemistry, University of Islamabad, Islamabad, Pakistan.

[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).

A. chasmanthum[2] Stapf ex Holmes and *A. heterophyllum*[3] Wall, which are abundantly available in Azad Kashmir, have had chemical examinations and a number of interesting diterpenoid alkaloids have been isolated from them,[4] no work seems to have been done on *A. violaceum* Jacq. An examination has now been made of the plant, which was collected in Neelam Valley, Azad Kashmir. The dried ground root was extracted with ethanol in a Soxhlet type extractor and the crude bases were isolated from the extract. These were freed from the acid and neutral components and separated into weak and strong bases.

From the weak base fraction, an alkaloid, m.p. 200–203° was isolated. Micro-analysis indicated the emperical formula for it to be $C_{34}H_{47}NO_{10}$. The NMR spectrum showed signals characteristic of one acetate group (3H, singlet at 8·7 τ), one ethyl group (3H, triplet at 8·89 τ, $J = 7{\cdot}0$ c/s) and four methoxyl groups (3H singlets at 6·44, 6·69, 6·74 and 6·82 τ), and five aromatic protons (multiplets between 1·9–2·2 τ). The highly shielded signal of the acetoxy protons indicated the presence of C-8–C-10 diester substitution.[5] From this data, we inferred that the alkaloid might be identical with indaconitine, an alkaloid which had already been isolated from *A. chasmanthum*. The identity of the alkaloid was established by the saponification of the alkaloid to yield *pseudoaconine*, $C_{25}H_{41}O_8N$, m.p. 88·90° (lit. m.p. 93–94°)[6] and benzoic acid. The comparison of the alkaloids with authentic samples of indaconitine and pseudoaconine, obtained from the dried roots of *A. chasmanthum* Stapf finally confirmed their identity.

During the isolation of indaconitine from *A. chasmanthum* Stapf, a considerable amount (10%) of colourless crystals was obtained, when the hot methanolic extract was allowed to stand at room temperature overnight; these were identified as sucrose by usual chemical tests.

EXPERIMENTAL

M.ps are uncorrected. IR spectra are of KBr discs and were taken on a Perkin–Elmer 337 grating spectro meter, NMR spectra were measured at 60 M/C in deuterio-chloroform with tetramethyl silane as external standard. TLC was carried out with the solvent system ether and benzene on glass plates coated with alumina.

Isolation of alkaloids. The air dried root (600 g) was ground to a powder and extracted with 5 × EtOH (10 l.) in a Soxhlet type extractor. The combined extracts were filtered, and concentrated to a thick syrup. It was dissolved in 2 N H_2SO_4 and the solution filtered. The filtrate was washed with Et_2O to remove acidic and neutral components; it was then made weakly basic with Na_2CO_3 and extracted with $CHCl_3$. Evaporation of extract left a residue of weak bases (15·0 g).

Isolation of indaconitine. The weak bases (15 0 g) were dissolved in benzene (50 ml) and chromatographed on a column of alumina (300 g). The column was eluted with benzene, benzene–$CHCl_3$, $CHCl_3$ and MeOH. Practically, all of the alkaloids were eluted with benzene. Fraction of 50 ml were collected and each fraction was evaporated under reduced pressure and the residue were examined by TLC. All benzene fractions showed a single identical spot and therefore were mixed together, evaporated to dryness and the residue crystallized from ether. A total quantity of 6·0 g of indaconitine, m.p. 200–203°, $[\alpha]_D$+18·3 (*c* 2 0% in EtOH), was obtained IR-(KBr) 3500 (OH), 2930, 2890, 2825, 1715 (ester carbonyl) 1445, 1360 (C-CH_3), 1600 (aromatic ring) 1275 (ester carbonyl) and 1110 cm^{-1} (hydroxyl). The NMR spectrum contained a triplet centered at τ 8·90 (N-ethyl), single at 8·70 (acetyl), 6·82 (3H), 6·74 (3H), 6·69 (3H), 6·44 (3H) indicating the presence of four methoxyl groups, a doublet at 5·1 (1H) and multiplets between 1·9–2·7 (5H, aromatic protons). (Found: C, 64·94; H, 7·44; N, 2·32; OMe 19·74%; Calc. for $C_{34}H_{47}NO_{10}$; C, 64·84; H, 7·52; N, 2·22; OMe, 19·71%).

Hydrolysis of indaconitine. Indaconitine (1 g) was dissolved in MeOH (25 ml) containing NaOH (100 mg) and H_2O (5 ml). The solution was refluxed 1 hr and the MeOH removed under reduced pressure. The residual solution was diluted with H_2O (25 ml) and extracted with $CHCl_3$ (5 × 40 ml). The extracts after

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drying (Na_2SO_4) were evaporated under reduced pressure to a crystalline mass. This was recrystallized from Et_2O to give colourless needles of pseudoaconine, m.p. 88–90° (lit. m.p. 93–94°). IR (KBr) 3400 (OH), 2860, 2810, 1450, 1370, 1100, 1030, 980 and 925 cm^{-1}. The NMR spectrum showed signal for a methyl group at τ 8·91 (triplet, $N-CH_3CH_2$) and four methoxyl groups at 6·76, 6·70 and 6·58 (12H). (Found: C, 62·15; H, 8·53; N, 3·01; OMe, 25·63% Calc. for $C_{25}H_{41}O_8N$: C, 62·11; H, 8·49; N, 2·99; OMe 25·67%). The alkaline, aqueous solution was acidified with HCl and continuously extracted with $CHCl_3$. The extract after drying and evaporation yielded benzoic acid, m.p. 120°, either alone or on admixture with an authentic sample.

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RUTACEAE

CHLOROFORM-SOLUBLE ALKALOIDS OF *FAGARA LEPRIEURII*

F. FISH and P. G. WATERMAN

Division of Pharmacognosy and Forensic Science, Department of Pharmaceutical Chemistry, University of Strathclyde, Glasgow C1, Scotland

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Abstract—The dried root and stem barks of *Fagara leprieurii* Engl. have yielded 1-hydroxy-3-methoxy-10-methylacridan-9-one, nitidine and chelerythrine together with the previously reported 1-hydroxy-2,3-dimethoxy-10-methylacridan-9-one and skimmianine.

INTRODUCTION

THE BARK of *Fagara leprieurii* Engl. (syn. *F. angolensis* Engl.) has previously been shown to contain the furoquinoline alkaloid skimmianine[1] and 1-hydroxy-2,3-dimethoxy-10-methylacridan-9-one.[2] Although angoline and angolinine have also been reported,[3,4] angoline has more recently been shown to be 9-methoxychelerythrine, an artefact of chelerythrine,[5] while angolinine is probably identical with nitidine.[6]

We now confirm the presence of the benzophenanthridine alkaloids, chelerythrine (IV) and nitidine (V) and report the occurrence of 1-hydroxy-3-methoxy-10-methylacridan-9-one

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