278. *Alkaloids in the Roots of* Rauwolfia perakensis.*

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From the roots of *Rauwolfia perakensis*, we have isolated in crystalline form sucrose, a phytosterol, ajmaline, isoreserpiline, reserpine, sarpagine, and two new alkaloids (pelirine and perakine). Three other, apparently new alkaloids have been isolated as salts.

Rauwolfia perakensis is a shrub that grows wild in the Northern States of Malaya. It has been cultivated at the Serdang Experimental Station of the Department of Agriculture, Federation of Malaya. From the roots of *R. perakensis* Chatterjee and her co-workers [1] had isolated γ-sitosterol, reserpine, and a methoxy-free indoline base which they named perakenine but later [2] considered to be rauwolfinine.[3] We have carried out a more complete investigation of the same plant.

The methods of separation used by Siddiqui and Siddiqui [4] and by Klohs *et al.*[5] were unsuccessful in our hands. Extraction with ethanol, followed by fractional precipitation

Fig. 1.

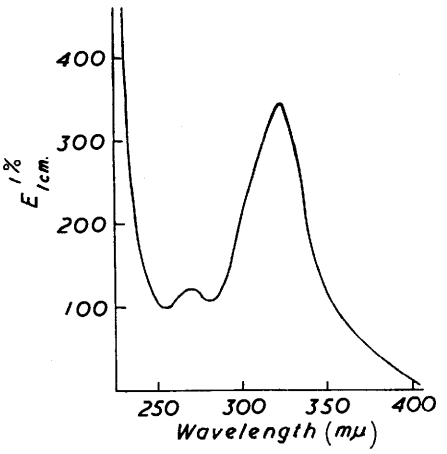
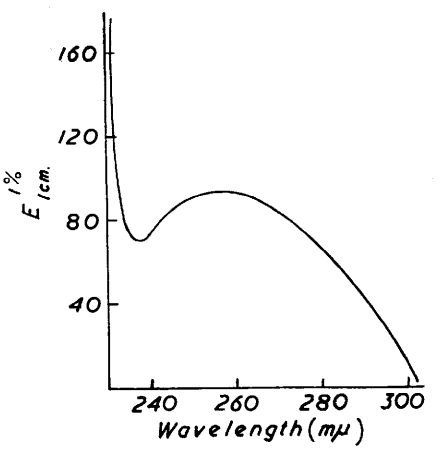


Fig. 2.



Ultraviolet spectra of (Fig. 1) *pelirine and* (Fig. 2) *perakine.*

and extraction, as detailed in the Experimental section, yielded sucrose [6] (2%) and nine alkaloids, namely, ajmaline,[4,7] isoreserpiline,[8] reserpine,[9] sarpagine,[10] two new crystalline bases named pelirine, perakine, and three apparently new amorphous bases designated RP-1, RP-2, and RP-3 which were isolated as crystalline salts.

The ultraviolet spectra (see Figures) of pelirine and perakine differentiate them from

* For a preliminary communication see Proc. UNESCO Symposium on Phytochemistry, Kuala Lumpur, UNESCO S.E.A. Science Co-operation Office, Djakarta, 1957, p. 181.

[1] Chatterjee and Talapatra, *Naturwiss.*, 1955, **42**, 182.
[2] Chatterjee, Pakrashi, and Werner, *Fortschr. Org. Naturstoffe*, 1956, **13**, 346.
[3] Chatterjee and Bose, *Science and Culture*, 1951, **17**, 139.
[4] Siddiqui and Siddiqui, *J. Indian Chem. Soc.*, 1931, **8**, 667.
[5] Klohs, Draper, Keller, Malesh, and Petracek, *J. Amer. Chem. Soc.*, 1954, **76**, 1332.
[6] Djerassi, Gorman, Nussbaum, and Reynoso, *J. Amer. Chem. Soc.*, 1954, **76**, 4664; Hochstein, Murai, and Boegemann, *J. Amer. Chem. Soc.*, 1955, **77**, 3551.
[7] Anet, Chakravarti, Robinson, and Schlittler, *J.*, 1954, 1242.
[8] Stoll, Hofmann, and Brunner, *Helv. Chim. Acta*, 1955, **38**, 270.
[9] Mueller, Schlittler, and Bein, *Experientia*, 1952, **8**, 338.
[10] Stoll and Hofmann, *Helv. Chim. Acta*, 1953, **36**, 1143; Bose, Talapatra, and Chatterjee, *J. Indian Chem. Soc.*, 1956, **33**, 379; Poisson and Goutarel, *Bull. Soc. chim. France*, 1956, 1703; Ishidate, Okada, and Saito, *Pharm. Bull. (Japan)*, 1955, **3**, 319.

previously recorded indole and indoline bases, and indeed they may not possess this skeleton. Pelirine, $C_{21}H_{28}O_4N_2$, has one methoxyl group and four active hydrogen atoms. Perakine, $C_{21}H_{22}O_3N_2$, is a stereoisomer of an alkaloid obtained from *R. vomitoria*,[11] and Dr. Hofmann in a personal communication has told us that both have one *C*-methyl and do not give crystalline salts, and that they have identical ultraviolet spectra; moreover, except in the finger-print region they have identical infrared spectra. A characteristic strong doublet was found at 5·8 and 5·9 μ but cannot yet be assigned.

Experimental

Microanalyses are by Dr. W. Zimmermann, Melbourne. Alumina was prepared by washing Merck's alumina with dilute sulphuric acid and then water until it was free from sulphate, drying and heating it at 150—160° for ~4 hr. For chromatography Whatman No. 1 paper was used, developing systems being (i) the aqueous phase of butan-1-ol–acetic acid–water (10 : 3 : 8) and (ii) the organic phase of pentyl alcohol–light petroleum (b. p. 80°)–acetic acid–water (3 : 1 : 3 : 3), the latter giving better separation.

Extraction.—The powdered root (3 kg.) was percolated with 95% ethanol until exhausted. The percolate was concentrated under reduced pressure in a cyclone evaporator.

Sucrose.—Sucrose crystallised from the cooled ethanolic concentrate (60 g., 2%). Purified by dissolution in the minimum quantity of water and addition of ethanol, it had m. p. 185°, $[\alpha]_D^{21}$ +63·3° (*c* 11·53 in H_2O).

Preliminary Fractionation of Total Alkaloids.—The ethanolic filtrate from the sucrose was poured into water. The liquid was decanted from the brown precipitate which was then extracted with water until the extract was nearly colourless and free from alkaloids. The insoluble material formed fraction I (37·2 g.).

The aqueous filtrates were combined and extracted with chloroform until no more alkaloid was taken up. After drying (Na_2SO_4), the extracts were evaporated under reduced pressure, yielding fraction II (19·2 g.).

The aqueous phase was then adjusted to pH 8·0 with sodium hydrogen carbonate solution, and the yellowish precipitate was collected, forming fraction III (40 g.).

The filtrate from fraction III was adjusted to pH 9·0 with sodium carbonate solution and extracted repeatedly with ethyl acetate until the aqueous phase was free from alkaloids. The extract was dried (Na_2SO_4) and evaporated to give fraction IV (21·8 g.).

Fraction I.—Fraction I (5 g.) was dissolved in chloroform containing a few drops of methanol and adsorbed on alumina. The solvents were removed *in vacuo* and the alumina placed on top of a column of alumina (200 g.) which was eluted as stated below, fractions of 100 ml. being collected.

Fractions 1—23 (benzene). The eluate yielded only a small amount of yellowish non-alkaloidal oil.

Fractions 24—34 (benzene–chloroform, 1 : 1). A non-alkaloidal yellowish residue (72 mg., 0·014%) was obtained on removal of the solvent. The substance crystallised from methanol as plates, m. p. 146—147°, and gave a strong Liebermann–Burchardt test purple ⟶ blue ⟶ green for sterols.

Fractions 42—54 (chloroform). The eluate showed an intense yellowish-green fluorescence in ultraviolet light. After evaporation, the reddish-brown residue was extracted with hot benzene from which on cooling a yellow material was precipitated. This gave colourless needles of reserpine (52 mg., 0·01%) when recrystallised from methanol. When further recrystallised this had m. p. and mixed m. p. 263—264° (278—279° *in vacuo*) (Found: C, 64·8; H, 6·7; N, 4·9. Calc. for $C_{33}H_{40}O_9N_2$: C, 65·1; H, 6·6; N, 4·6%). It gave with Fröhde's reagent the characteristic colour change (yellow to green to blue) and had the correct infrared spectrum. In dilute acetic acid it gave with dilute hydrochloric acid the hydrochloride, forming needles, m. p. 223—224°, from ethanol (Found: C, 59·7; H, 6·4; N, 5·0. Calc. for $C_{33}H_{40}O_9N_2,HCl,H_2O$: C, 59·8; H, 6·5; N, 4·2%). It similarly gave a sulphate, m. p. 263—264° (from methanol) (Found: C, 56·2; H, 6·2; N, 3·8. $C_{33}H_{40}O_9N_2,H_2SO_4$ requires C, 56·1; H, 6·0; N, 4·0%).

Fraction II.—Fraction II (19·2 g.) was dissolved in a small volume of methanol and poured

[11] Hofmann and Frey, *Helv. Chim. Acta*, 1957, **40**, 1866.

with constant stirring into nine times its volume of ether. The flocculent brown precipitate was extracted with further quantities of ether containing 10% of methanol. The ethereal extract was washed with sodium hydrogen carbonate solution, and the washings were shaken again with ether. The combined ether extracts were dried (Na_2SO_4) and evaporated under reduced pressure to give a light brown fraction II-t (7·6 g.). The ether-insoluble residue was devoid of alkaloids.

Fraction II-t (5·4 g.) was chromatographed in the minimum amount of chloroform on alumina (250 g.) and eluted (see below) in fractions of 50 ml. Each eluate was taken to dryness, weighed, and chromatographed on paper, and suitable fractions were combined. The residues from each group of fractions were dissolved in the minimum amount of methanol and allowed to crystallise. When the alkaloids failed to crystallise, aliquot portions of the solutions were treated with hydrochloric, sulphuric, nitric, and picric acid. The following materials were thus obtained.

Fractions 5—8 (benzene–chloroform 3 : 1). *Alkaloid* RP-1 *hydrochloride* (113 mg., 0·005%) recrystallised from aqueous methanol, then having m. p. 192—194° (decomp.) (Found: C, 58·4; H, 6·9; N, 6·0; Cl, 8·2. $C_{22}H_{26}O_4N_2$,HCl,CH_3·OH,H_2O requires C, 58·8; H, 7·1; N, 6·0; Cl, 7·5%).

The *nitrate* was similarly isolated and crystallised from aqueous methanol or aqueous ethanol as solvated prisms, m. p. 240—242° (decomp.), which became opaque and powdery when dried (Found, for crystals from aqueous methanol: C, 57·9; H, 6·25; N, 8·8. $C_{22}H_{26}O_4N_2$,HNO_3,CH_3·OH requires C, 57·85; H, 6·55; N, 8·8. Found, for crystals from aqueous ethanol: C, 58·8; H, 6·7; N, 8·55. $C_{22}H_{26}O_4N_2$,HNO_3,C_2H_5·OH requires C, 58·6; H, 6·8; N, 8·55%). The *picrate*, prepared from the nitrate, crystallised from ethanol in yellow needles, m. p. 204—205° (Found: C, 55·0; H, 4·9; N, 11·4. $C_{22}H_{26}O_4N_2$,$C_6H_3O_7N_3$ requires C, 55·0; H, 4·8; N, 11·5%). The free base was amorphous (m. p. 140—144°); it gave with Keller's reagent an emerald green colour which changed to pale green, and with concentrated nitric or sulphuric acid or Fröhde's reagent a yellow colour.

Fractions 9—15 (benzene–chloroform 3 : 1). Although the residues from these fractions crystallised from methanol, it was found more convenient to isolate the alkaloid as *nitrate* which (540 mg., 0·025%) crystallised as sparingly soluble needles, m. p. 252—254° (decomp.), from aqueous methanol (Found: C, 58·1; H, 6·2; N, 8·7. $C_{23}H_{28}O_5N_2$,HNO_3 requires C, 58·1; H, 6·15; N, 8·8%). The free base, isoreserpiline, was obtained as needles from the nitrate by means of ammonia; recrystallised from aqueous methanol, it had m. p. 211—212°, $[\alpha]_D^{23}$ −33° (*c* 0·77 in EtOH) (Found: C, 66·95; H, 6·8; N, 6·7; OMe, 22·2. Calc. for $C_{23}H_{28}O_5N_2$: C, 67·0; H, 6·8; N, 7·0; 3OMe, 22·6%), $\lambda_{max.}$ (in EtOH) 229 (log ε 4·49) and 304 mμ (log ε 3·91), $\lambda_{min.}$ 277 mμ (log ε 3·61). With Keller's reagent it gave a rose-red colour changing to light purple. The identity of the alkaloid [8] was confirmed by its infrared spectrum. With aqueous-methanolic hydrochloric acid it gave a *hydrochloride*, needles (from water), m. p. 250—252° (decomp.) (Found: C, 59·6; H, 6·7; N, 5·7; Cl, 7·0. $C_{23}H_{28}O_5N_2$,HCl,H_2O requires C, 59·5; H, 6·7; N, 6·0; Cl, 7·6%).

Fractions 17—27 (benzene–chloroform 3 : 1). These eluates gave *alkaloid* RP-2 *picrate*, (obtained from acetic acid) which from ethanol crystallised in yellow needles (178 mg., 0·008%), m. p. 136—138° (Found: C, 56·6; H, 4·9; N, 11·4; OMe, 3·5. $C_{24}H_{28}O_4N_2$,$C_6H_3O_7N_3$ requires C, 56·5; H, 4·9; N, 11·0; 1OMe, 4·9%). The base recovered from the picrate was amorphous (m. p. 76—80°).

Fractions 33—46 (benzene–chloroform 1 : 1). From these residues perakine partly crystallised (497 mg., 0·023%). It sublimed at 170°/0·40 mm. but was best purified by recrystallisation from acetone, forming needles, m. p. 185—186°, $[\alpha]_D^{23}$ −107° (*c* 0·98 in EtOH). On exposure to air, the crystals became opaque [Found: C, 71·5, 71·8, 71·6; H, 6·3, 6·3, 6·4; N, 7·8, 7·9; O, 14·2; OMe, 0; active H, 0·16; *C*-Me, 5·3; *N*-Me, 0·2%; *M* (Rast), 301. Calc. for $C_{21}H_{22}O_3N_2$: C, 72·0; H, 6·3; N, 8·0; O, 13·7; 1 active H, 0·28; 1*C*-Me, 4·25%; *M*, 350]. It gave no colour with concentrated sulphuric or nitric acid, ceric sulphate, or Fröhde's, or Keller's, or Adamkiewicz's reagent, and no crystalline salts.

Fractions 77—91 (benzene–chloroform 1 : 3). These eluates, in dilute acetic acid, gave *alkaloid* RP-3 *picrate*. Originally yellow, this changed on a boiling-water bath, to dark-red. Recrystallisation from aqueous ethanol gave dark-red needles (907 mg., 0·042%), m. p. 167—168°, becoming orange on drying and red again on exposure to air. For analysis they were dried to constant weight at 80° over phosphoric oxide in a vacuum (loss of wt., 5·37%. $2H_2O$

requires 5·1%) (Found: C, 53·2; H, 5·1; N, 10·4; OMe, 14·1. $C_{24}H_{32}O_6N_2,C_6H_3O_7N_3$ requires C, 53·5; H, 5·2; N, 10·4; 3OMe, 13·8%). The amorphous base (m. p. 110—114°) gave a wine-red colour with Fröhde's reagent, a brown colour changing to orange with concentrated nitric acid, and a yellow colour with concentrated sulphuric acid.

Fractions 99—107 (chloroform–methanol 1%). Reserpine crystallised (108 mg., 0·005%). Recrystallised from methanol, it had m. p. and mixed m. p. 261—262° (278—279° *in vacuo*). It gave reserpine hydrochloride, m. p. 223—224°.

Elution of the column with chloroform containing greater quantities of methanol yielded small amounts of reddish-brown residues which failed to give crystals.

Fraction III.—This fraction was only partly soluble in ether, chloroform, ethyl acetate, or ethylene dichloride. Like fraction II, it contained much resin and non-alkaloidal matter. The fraction (100 g.) was treated in the same way as fraction II to give fraction III-t (24 g.), a portion of which (20·5 g.) was suspended in chloroform (incomplete solution) and developed on alumina (500 g.). Fractions of 50 ml. were collected. The alkaloids could not be eluted by chloroform, but chloroform–methanol was effective.

Fraction 2—48 (chloroform–methanol 99 : 1). The residues were extremely soluble in methanol but gave *pelirine sulphate* (4·06 g., 0·06%). Suspending the sulphate in hot methanol and adding water gradually until complete solution took place, afforded, on cooling, yellow needles, m. p. 223—224° (decomp.) (Found: C, 58·8; H, 7·05; N, 6·5; O, 22·8. $C_{21}H_{28}O_4N_2,\frac{1}{2}H_2SO_4,\frac{1}{2}H_2O$ requires C, 58·6; H, 7·0; N, 6·5; O, 22·3%). Free *pelirine* crystallised from aqueous methanol in pale yellow plates, m. p. 130—131°, $[\alpha]_D^{22}$ −121° (*c* 1·0 in EtOH) (Found: C, 68·1; H, 7·4; N, 7·6; active H, 1·07; OMe, 8·4. $C_{21}H_{28}O_4N_2$ requires C, 67·9; H, 7·6; N, 7·5; 4 active H, 1·08; 1OMe, 8·3%). It gave with concentrated nitric acid a yellow colour changing to orange, with Fröhde's or Erdmann's reagent an orange colour, and with Keller's reagent a deep orange colour. The *picrate* crystallised from ethanol in yellow needles, m. p. 159—159·5° (Found: C, 54·0; H, 5·5; N, 10·8; OMe, 5·8. $C_{21}H_{28}O_4N_2,C_6H_3O_7N_3,C_2H_5{\cdot}OH$ requires C, 53·8; H, 5·7; N, 10·8; 1OMe, 4·8%).

Fractions 165—246 (chloroform–methanol 95—90 : 10). Ajmaline (0·92 g., 0·014%) crystallised slowly as the methanol solution was allowed to evaporate and recrystallised from methanol as stout prisms, m. p. 158—159°, $[\alpha]_D^{22}$ +133° (*c* 0·997 in $CHCl_3$) (Found, after drying at 100° *in vacuo*: C, 71·6; H, 8·0; N, 8·3; O, 11·75. Calc. for $C_{20}H_{26}O_2N_2,\frac{1}{2}CH_3{\cdot}OH$: C, 71·9; H, 8·2; N, 8·2; O, 11·7%). It gave a blood-red colour with concentrated nitric acid, slowly became pink with Erdmann's reagent, and gave with Keller's reagent a yellow colour changing to orange and then red. The identity of the alkaloid was confirmed by a mixed m. p. determination and chromatography.

Fraction 271—318 (chloroform–methanol, 75—50 : 25—50). Sarpagine (515 mg., 0·008%), crystallised from the methanolic solution, identical with that described in the next paragraph.

Fraction IV.—Fraction IV (21·8 g.) was taken up in methanol and left in a refrigerator overnight. The sarpagine which separated was washed with methanol. The alkaloid (2·67 g., 0·089%) was sparingly soluble in methanol. Recrystallised from a large quantity of methanol, it had m. p. 374° (*in vacuo*), $[\alpha]_D^{20}$ +55° (*c* 0·75 in pyridine); methanol of crystallisation was lost at 80° *in vacuo* and on sublimation at 260°/0·4 mm. (Found, for solvate: C, 70·5; H, 7·6; N, 8·6. Calc. for $C_{19}H_{22}O_2N_2,CH_3{\cdot}OH$: C, 70·15; H, 7·65; N, 8·2. Found, for anhydrous alkaloid: C, 73·45; H, 7·2; N, 9·0; O, 10·3. Calc. for $C_{19}H_{22}O_2N_2$: C, 73·5; H, 7·1; N, 9·0; O, 10·3%); the ultraviolet spectrum showed maxima at 277 (log ε 4·32), 280 (log ε 3·98), a shoulder at 291 mμ (log ε 3·82), and a minimum at 250 mμ. Its ultraviolet and infrared spectra were identical with those of authentic sarpagine.

The alkaloid reduced cold ammoniacal silver nitrate but Fehling's solution only on heating. It was soluble in dilute sodium hydroxide, the solution becoming green in air. It gave with Keller's reagent an intense violet colour changing to dark brown, and with Fröhde's reagent a deep blue colour. The hydrochloride was prepared from hot 0·5N-hydrochloric acid and, recrystallised from ethanol, decomposed at ~220° (Found, in material dried at 115° over P_2O_5 *in vacuo*: C, 64·9; H, 6·8; N, 7·7; O, 10·7; Cl, 10·2. Calc. for $C_{19}H_{22}O_2N_2,HCl,\frac{1}{4}H_2O$: C, 65·0; H, 6·7; N, 8·0; O, 10·25; Cl, 10·05%). The hydrobromide decomposed at about 220° (Found, after drying: C, 57·3; H, 6·0; N, 6·85; O, 9·6; Br, 19·9. Calc. for $C_{19}H_{22}O_2N_2,HBr,\frac{1}{4}H_2O$: C, 57·65; H, 6·0; N, 7·1; O, 9·1; Br, 20·2%).

The mother-liquors from sarpagine, when evaporated, gave a very dark residue. This was treated in the same manner as fraction III. By chromatography on alumina and elution with

chloroform containing increasing quantities of methanol, pelirine (0·023%), ajmaline (0·008%), and sarpagine (0·001%) were isolated in eluates parallel to those from fraction III-t.

We thank Mr. E. F. Allen, Mr. Lee Sin Fook (Department of Agriculture), and Mr. A. H. Millard (Department of Chemistry, Federation of Malaya) for supplies of roots of *Rauwolfia perakensis*, Messrs. Smith, Kline and French Laboratories, Philadelphia, for the infrared spectra, and Ciba Research Laboratories, Basle, Dr. A. Hofmann of Sandoz, Basle, Professor R. Goutarel of the Faculty of Pharmacy, Paris, Professor M. Ishidate of Tokyo University, Dr. M. W. Klohs of Riker Laboratories, Los Angeles, and Dr. S. K. Talapatra of the College of Science and Technology, Calcutta, for samples of *Rauwolfia* alkaloids.

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