STUDIES IN THE ALKALOIDS OF RAUWOLFIA CAFFRA SONDER

Part I.—Isolation of Ajmalicine, Ajmaline, Raucaffrine* and Three New Alkaloids, Raucaffricine, Raucaffriline and Raucaffridine

NOORUL HAQ KHAN, MOHAMMAD ATAULLAH KHAN AND SALIMUZZAMAN SIDDIQUI

Central Laboratories, Pakistan Council of Scientific and Industrial Research, Karachi

(Received September 17, 1964)

Rauwolfia caffra Sonder is known to be widely distributed in Central, East and South Africa. It has also been grown successfully under an experimental plantation project sponsored by the Pakistan Council of Scientific and Industrial Research in East Pakistan. The species is now considered to include *R. natalensis* Sond. Botanically *R. caffra* and *R. natalensis* Sond. are taken as conspecific and under the name *R. caffra* Sond; *R. inebrians* K. Schum is regarded as a synonym.[1] Because of the widespread distribution of the species in Africa it seems likely that the synonymy should be even more extensive. *R. obliquinervis* Stapf and *R. goetzei* Stapf presumably also belong here (under *R. caffra* Sond.) and *R. welwitschii* Stapf and *R. ochrosioides* K. Schum are only doubtfully distinct.

No work on the chemical constituents of the plant seems to have been carried out till Rindl and Groenewood[2] in 1932, working on the bark of *R. natalensis* (*R. caffra*), isolated two uncrystallised alkaloidal fractions, one of which gave the blood-red colour reaction with nitric acid, characteristic of dihydroindole derivatives.

In the same year Koepfli [3] reported the isolation of a crystalline alkaloid from the bark of *R. caffra* to which he assigned the molecular formula $C_{20}H_{26}O_3N_2$, on the basis of the analysis of the base and its halogen salts. This base was quite distinct from ajmaline, giving indications of a quarternary character, and was named rauwolfine.** He further reported the isolation of two insufficiently characterized crystalline bases A and B, the former of which has been recorded as showing m.p. 294.95°C. (uncorr.).

More recently Schuler and Warren[4] have reported the isolation of ajmaline and reserpine from *R. natalensis* (*R. caffra*).

Following the studies in the isolation of alkaloidal complexes from the fresh undried roots of *R. serpentina* through a technique of dialysis with organic solvents, it was considered of interest to reinvestigate the constituents of *R. caffra* using freshly harvested, undried plant material. As a result of studies in the roots and root bark of the plant, obtained through air transport from an experimental plantation in East Pakistan, and also from South Africa, the following products have been isolated:—

(i)	Raucaffrine (Identified as perakine)	$C_{21}H_{22}O_3N_2$.
(ii)	Raucaffridine	$C_{21}H_{24}O_3N_2$.
(iii)	Ajmalicine	$C_{21}H_{24}O_3N_2$. $1\frac{1}{2}H_2O$.
(iv)	Ajmaline	$C_{20}H_{26}O_2N_2$.
(v)	Raucaffricine	$C_{26}H_{32}O_8N_2$. $\frac{1}{2}H_2O$.
(vi)	Raucaffriline	$C_{21}H_{22}O_3N_2$.
(vii)	Serposterol	$C_{30}H_{48}O_2$.
(viii)	Caffrosterol (An uncharacterised sterol)	$C_{20}H_{34}O_2$.

It may be noted that the base, Raucaffridine, was obtained only once in the first working of the sample from South Africa, and it was not possible for the present authors to isolate Koepfli's rauwolfine from either of the two samples.

The procedure adopted by Siddiqui[5] in the isolation of the various alkaloidal complexes of *R. serpentina* from its alcoholic dialysates were not found practicable for the isolation of the individual alkaloid in the present case as most of the alkaloidal products remained insoluble in amyl alcohol. In the case of *R. serpentina* most of the

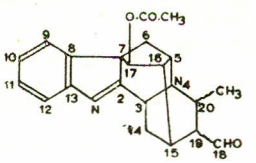
*At the time this work was in hand, Kiang and Wan, J. Chem. Soc., 1396 (1960) had also been successful in isolation of raucaffrine, which they named as perakine, from another pecies of Rauwolfia, *R. perakensis*. The identity of the two bases has been established in the present work.

This name should not be confused with the base isolated by van Itallie and Steenhauer (Arch. Phrim; 1932, **270, 313), also named as rauwolfine, though it was obviously identical with ajmaline reported earlier (Siddiqui and Siddiqui, J. Indian Chem. Soc., 1931, **8**, 667).

alkaloids could be shaken out of the aqueous solution with amyl alcohol, and the same procedure also proved effective in the isolation of alkaloidal complexes from *R. vomitoria.*[6] This might be indicative of the fact that in contrast to *R. serpentina* and *R. vomitoria* the alkaloids do not occur in the form of complexes in *R. caffra*

Raucaffrine (perakine) and raucaffridine were initially obtained by taking advantage of their varying solubility in various organic solvents. Subsequently, raucaffrine (perakine) was also obtained by subjecting the benzene soluble portion of the base to column chromatography (Brockmann's Grade Alumina) and raucaffridine from the benzene insoluble portion of the base in the manner described in the experimental part.

As the physical constants of raucaffrine appeared to be nearest to those of perakine, it was considered desirable to study the alkaloid raucaffrine with the aid of NMR, which showed that perakine and raucaffrine are identical. Perakine has been assigned the following structure,[7] which represents the first recognition of the indolenine alkaloid in any species of Rauwolfia.

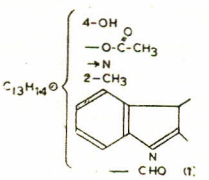


The separation of raucaffricine and raucaffriline was mainly based on differences in the strength of these bases and the solubility of their hydrochlorides. While the weak bases, ajmalicine and raucaffriline were obtained from the ethyl acetate soluble fraction of the total alcoholic extractive, the other crystalline bases like raucaffricine, raucaffrine (perakine) and ajmaline were obtained from the water soluble hydrochlorides of the aqueous solution of the total extractive. A little amount of raucaffricine was also obtained from the water-insoluble hydrochlorides, the latter forming about 50 percent of the total alkaloids.

On a careful check up of the analytical data, the melting points, colour reactions and other physical properties, it appears that raucaffricine and raucaffriline are not identical with any of the known bases and form an addition to the already long list of alkaloids isolated from the various Rauwolfia species. Although ajmalicine m.p. 250-252°C. and tetrahydro-alstonine m.p. 228-230°C. have the same molecular formula ($C_{21}H_{24}O_3N_2$) as raucaffridine; the melting points of the former bases differ markedly from the latter (221°C.). The infra-red spectrum shows that raucaffridine has no twin peaks at 6μ region, characteristic of the unsaturated ester grouping ($CH_3OOC\text{-}C^{I}=$) which is present in ajmalicine and tetrahydro-alstonine.

Raucaffricine, seems to be the major alkaloid of *Rauwolfia caffra* root bark (yield* 0.17%) whereas raucaffrine (perakine) is the major alkaloid of *Rauwolfia caffra* thin roots (yield 0.11%).

Raucaffricine which analyses for $C_{26}H_{32}O_8N_2$, is monoacid base and tertiary in character, forming a crystalline monomethiodide. Its I-R spectrum indicated that it is a poly-hydroxy base, and the formation of tetra-acetyl and tetrabenzoyl derivatives showed that it contains four hydroxyl groups. The presence of an acetyl group in raucaffricine was shown by I-R spectrum and micro analytical values of one acetyl in the base and five in its tetra-acetyl derivative. It reduces Fehling's and ammoniacal silver nitrate solutions, indicative of a possible aldehyde grouping, but this could not be confirmed through I-R or the formation of oxime and phenylhydrazone derivatives. U.V. spectrum in alcohol indicates raucaffricine to be an indolenine derivative ($\lambda_{Max.}$ 219.5, 258 mμ; $\lambda_{Min.}$ 236mμ). Raucaffricine can therefore be written as:



The other new alkaloid, raucaffriline, crystallises in cubes from ethyl acetate and melts at 200-201°C. It analysed for $C_{21}H_{22}O_3N_2$, having 1 N-CH_3, 2 C-CH_3, one active H and no O-CH_3.

Experimental

Isolation of Raucaffrine (Perakine), Ajmaline and Raucaffridine

Eight pounds of fresh roots of *Rauwolfia caffra* Sonder from East Pakistan (about 3½ lbs. dry weight) were chopped into small pieces and repeatedly percolated with alcohol (three times) and the solvent from the combined percolatesw ere removed *in vacuo*. The semisolid residue (500 g.) thus obtained was digested with warm distilled water and the decantates strained through cotton

* All the yields have been calculated on dry weight basis.

wool after bringing their pH from 5 to 6.5 with dilute ammonia. It was then charcoaled, and the base liberated from the clear brownish filtrate with ammonia (30%) was exhaustively extracted with ethyl acetate. The ethyl acetate solution was washed with water, dried over anhydrous sodium sulphate and freed of the solvent, when it gave 25 g. of a semisolid basic residue. A further quantity of the base (5 g.) was obtained by extracting the insoluble portion of the total alcoholic extract with dilute acetic acid and working up the solution in the manner described above.

Raucaffrine.—The crude total base (30 g.) was exhaustively extracted with benzene in the hot and the solution treated with a little petroleum ether which precipitated off darkish material that was neglected. The clear light coloured solution obtained on filtration was passed through a column of alumina (Aluminium oxide "woelm" activity grade, M. Woelm-Eschwege; Fabrik Chemisch-Pharmazeutischer Praparate) and eluted with benzene; 75 ml. fractions of the eluate were collected, fraction No. 2 was slightly yellow; Nos. 3, 4 and 5 were reddish brown and the subsequent fractions were straw yellow. On removal of the solvent from fractions 2,3,4 and 5 and treating the residues with a little ethyl acetate and petroleum ether a crystalline base *raucaffrine* (perakine) was obtained as glistening rods. The fractions were combined and the base (4 g.) on repeated crystallisation melted at 186-189°C.

Raucaffrine (perakine) was also isolated from the mother liquor of raucaffricine, by removing the solvent *in vacuo* and working up the residue according to the procedure described above. With this method an yield of 0.11% raucaffrine was obtained from the thin roots of *Rauwolfia caffra.*

Ajmaline.—The column was subsequently eluted with ethyl acetate and then with methanol. The ethyl acetate eluate gave cream coloured amorphous bases, but the methanol eluate yielded a small quantity of a base m.p. 158°C., which could be identified with ajmaline through its mixed m.p. with an authentic sample of the base.

Raucaffridine.—The benzene insoluble fraction of the total base was digested with ethyl acetate. The ethyl acetate soluble portion gave the ajmaline test with nitric acid, but failed to yield any crystalline base. The ethyl acetate insoluble portion was taken up in methanol and treated with a little ethyl acetate which precipitated off a darkish sticky material. On keeping the clear filtered solution over night at room temperature, after the addition of some more ethyl acetate, *raucaffridine* (m.p. 221° C. decomp.) crystallised in fine short needles. It analysed for $C_{21}H_{24}O_3N_2$. Found: C, 71.45; H, 7.20; O, 13.27; N, 8.08; MW, 340. Calculated for $C_{21}H_{24}O_3N_2$: C, 71.57; H, 6.86; O, 13.62; N, 7.95; MW, 352.

ISOLATION OF RAUCAFFRICINE, AJMALICINE AND RAUCAFFRILINE

In another working, fresh undried root *bark* of *Rauwolfia caffra* (7.75 kg.; dry weight, 2.17 kg.) was percolated five times with ethanol. The total percolate was concentrated in a cyclone evaporator at 20-25°C. The concentrate was freed of the solvent under reduced pressure below 50°C. The residue thus obtained (376 g., 17.3%), was digested with hot water (Ca. 2.5 litres) and distributed into aqueous and ethyl acetate layers. A small quantity of non-basic material (23 g.) did not go into either of these solvents and was neglected. The water and ethyl acetate soluble fractions were worked up as follows to yield raucaffricine, raucaffrine, ajmalicine, raucaffriline and the two sterols (serposterol and caffrosterol).

Raucaffricine.—The well cooled aqueous layer was saturated with sodium chloride, and the water insoluble hydrochloride formed was filtered. The filtrate was basified with 10% ammonia and repeatedly extracted with ethyl acetate. The ethyl acetate solution (Ca. 1 litre) was washed with water, dried over Na_2SO_4 (anhydrous) and filtered. On keeping overnight, the solution gave a crystallisate which on recrystallisation from a mixture of moist ethyl acetate and MeOH (5:1) finally yielded *raucaffricine* as hexagonal plates m.p. 220°C with initial frothing at 186°; yield 1 g., 0.046%. Working with another lot of the material, the yield obtained was much higher—0.17%.

As already stated earlier, the mother liquor of raucaffricine yielded raucaffrine. From the water, insoluble hydrochlorides, no crystalline alkaloid, except for a small quantity of raucaffricine, could be obtained.

The ethyl acetate soluble fraction of the total alcoholic extractive (31.7 g.) was digested with petroleum ether (b.p. 60-80°) and the insoluble residue (15.5 g.) was divided into ether soluble and ether insoluble fractions. The ethereal solution was treated with a little peroleum ether, filtered, and freed of the solvent. The residue was taken up in methanol and kept overnight at room temperature, after adding a little ethyl acetate, when *ajmalicine* crystallised out (yield 0.22 g., 0.01%) m.p. 253°C. It gave no depression in m.p. with an authentic sample of ajmalicine.

Raucaffriline.—On adding ether to the mother liquor of ajmalicine a small amount of blackish material separated out, that was filtered off. The filtrate was extracted out with 10% acetic acid and the acidic solution basified with 30% ammonia and the liberated base extracted out with ethyl acetate. The ethyl acetate was washed, dried and freed of the solvent *in vacuo*, yielding 3.8 g. of white amorphous bases, which were divided into ether soluble and ether insoluble fractions. The ether soluble part was chromatographed over a column of alumina, developed with petroleum ether. The column was first eluted with ether, then with ethyl acetate and finally with methanol. The ethyl acetate eluate yielded a light yellow residue which was crystallised from a mixture of ethyl acetate and petroleum ether to give 180 mg. of *raucaffriline*. On repeated crystallisation from ethyl acetate it finally melted at 200-201°C. It is soluble in alcohol, methanol, ethyl acetate, sparingly soluble in ether and insoluble in petroleum ether. It failed to yield any crystalline salt. It analysed for $C_{21}H_{22}O_3N_2$. Found: C, 71.91; 72.04; H, 6.57; 6.41; O, 13.70; 13.83; N, 8.01, 8.03; C-CH_3, 10.92; active H, 0.35; MW, 350. Calculated for $C_{21}H_{22}O_3N_2$ C, 71.98; H, 6.33; O, 13.70; N, 8.00; 2 C-CH_3, 8.57; 1 active H, 0.35; MW, 350.

Sterols.—*Serposterol* $C_{30}H_{48}O_2$ (0.45 g.) melting at 160°C. was obtained from the unsaponifiable portion of the petroleum ether soluble part of the ethyl acetate soluble fraction of the total alcoholic extractive.

Another non-basic crystalline product was obtained from the acetic acid insoluble portion of the ajmalicine mother liquor and on repeated crystallisation with methanol finally gave crystalline needles (131 mg.) which melted at 297° (decomp.) and analysed for $C_{20}H_{34}O_2$. Found: C, 78.40; H, 10.99; O, 10.34 and MW, 298. Calculated for $C_{20}H_{34}O_2$: C, 78.38; H, 11.18; O, 10.44 and MW, 306.

It gave Liebermann sterolic test and has been provisionally named as *caffrosterol.*

CHARACTERISATION OF RAUCAFFRINE AND ITS IDENTIFICATION WITH PERAKINE*

Raucaffrine analysed for $C_{21}H_{22}O_3N_2$ Found: C, 71.61; H, 6.31; O, 14.02; N, 7.92; MW, 395, 325. Calculated for $C_{21}H_{22}O_3N_2$: C, 71.98; H, 6.33; O, 13.7; N, 8.0; MW, 350 $[\alpha]_D$ +120° ($CHCl_3$).

NMR STUDIES ON RAUCAFFRINE AND PERAKINE

	Raucaffrine (δ)	Perakine (δ)
Aromatic (4 protons)	Multiplet 7.2-7.75	Multiplet 6.91-7.60
C(3)-H	Distroted quartet 4.20	Quartet centred 4.21
C(17)-H	Doublet 4.97	Doublet centred 4.97
Me of OAc (3 protons)	2.17	2.17
C-Me	Doublet centred 1.29 (J 6.27)	Doublet centred 1.42 (J 6. 3)

The NMR data were obtained on deutero-chloroform solutions with a Varian Model HR60 spectrometer at 60 mc/sec. with tetramethyl-silane acting as internal standard. The IR spectra of raucaffrine and perakine are identical and the melting point is not depressed on admixture of the two.

*Raucaffrine Picrate**.—The picrate crystallised out in fine short needles on treating the alcoholic solution of raucaffrine with a saturated alcoholic solution of picric acid in the hot and allowing it to cool down at room temperature. On repeated recrystallisation from alcohol it finally showed m.p. 162°C. (decomp.).

*Raucaffrine Methiodide**.—Raucaffrine (100 mg.) was dissolved in chloroform and kept over night at room temperature with 1 ml. of methyl iodide. Raucaffrine methiodide was obtained as a slightly yellow mass on removal of the solvent *in vacuo*, which crystallised in needles from methanol m.p. 208-9°C. (decomp.).

CHARACTERISATION OF RAUCAFFRICINE

Raucaffricine is soluble in alcohol and methanol,

*Taken from Ph.D. thesis of N.H. Khan at London Uninversity (1963), with the kind permission of Dr. L. K. Sharp.

*These two salts of the corresponding perakine have not been reported earlier.

sparinghly soluble in ethyl acetate and insoluble in other bench solvents. It analysed for $C_{26}H_{32}O_8N_2.\frac{1}{2}H_2O$, after drying to constant weight at room temperature over P_2O_5 *in vacuo*. $[\alpha]_D^{30}$ + 14.5° (C_2H_5OH). Found: C, 61.03; H, 6.39; O, 27.22; N, 5.63; MW, 513. Calculated for $C_{26}H_{32}O_8N_2.\frac{1}{2}H_2O$: C, 61.29; H, 6.48; O, 26.72; N, 5.50 and MW, 509. Raucaffricine dried at 100°C. to constant weight over P_2O_5 *in vacuo* analysed for $C_{26}H_{32}O_8N_2$, 6 active H, 2 C-CH3 and no O-CH3. Found: C, 61.84; H, 6.30; O, 26.04; N, 5.71; active H, 1.2; C-CH_3, 5.97; Acetyl value, 9.09% and MW, 506. Calculated for $C_{26}H_{32}O_8N_2$: C, 62.39; H, 6.44; O, 25.57; N, 5.60; 6 active H, 1.2; 2 C-CH_3, 6.00; Acetyl value for one acetyl group, 8.6% and MW, 500.

Raucaffricine Methiodide.—Raucaffricine (100 mg.) was dissolved in methanol (2 ml.) and kept over night in the cold with methyl iodide (1 ml.). The solvent was removed *in vacuo* and the methanolic solution of the residue (125 mg.) was treated with a little ethyl acetate when raucaffricine methiodide separated as an oil, which crystallised in prisms m.p. 200°C. (decomp.) on standing for two days. It analysed for $C_{26}H_{32}O_8N_2.CH_3I$. Found: C, 50.49; H, 5.55; N, 3.97; I, 19.60 and N-CH_3, 2.60. Calculated for $C_{26}H_{32}O_8N_2.CH_3I$: C, 50.46; H, 5.45; N, 4.36; I, 19.77 and I N-CH_3, 2.33.

Raucaffricine Tetra-acetate.—Raucaffricine (100 mg.) was dissolved in pyridine (1 ml.), treated with acetic anhydride (1 ml.) and kept over night at room temperature. On removal of the solvent *in vacuo* a glassy residue was obtained which crystallised from a mixture of ethyl acetate and petroleum ether in cubes (100 mg.). On recrystallisation from ethyl acetate it showed m.p. 234°C. and analysed for $C_{34}H_{40}O_{12}N_2$. Found: C, 61.15; H, 5.91; O, 28.33; N, 4.56; Acetyl value, 31.88; 32.52 and MW, 642. Calculated for $C_{34}H_{40}O_{12}N_2$ C, 61.08; H, 5.98; O, 28.74; N, 4.19; Acetyl value for 5 acetyl groups, 32.18 and MW, 668.

Raucaffricine Tetra-benzoate.—To a solution of raucaffricine (100 mg.) in pyridine (1 ml.) was added benzoyl chloride (1.0 ml.), and the reaction mixture was kept over night at room temperature when a dark red colour developed. Raucaffricine tetrabenzoate was obtained as a brown semisolid mass on shaking the reaction mixture with a small quantity of distilled water. On repeated crystallisation from methanol it formed colourless silky needles (100 mg.) m.p. 278-80°C. (decomp.) and analysed for $C_{54}H_{48}O_{12}N_2$. Found after drying at 100°C. to constant weight over P_2O_5 *in vacuo*: C, 70.66; H, 5.13; O, 21.09 and N, 3.37. Calculated for $C_{54}H_{48}O_{12}N_2$: C, 70.74; H, 5.24; O, 20.96 and N, 3.06.

Acknowledgement.—The authors feel grateful to Dr. S. Hedayetullah, and Professor J.M. Watt for generous supplies of *R. caffra* from East Pakistan and South Africa, respectively, and to Professor A. Kiang who very kindly provided a sample of perakine for comparison. The authors are also indebted to Mr. M. Ameem for assistance in some of the experimental work.

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