

### Summary

*Sym.*-2,2'-dimethylbenzopinacol and *sym.*-2,2'-dimethoxybenzopinacol have been rearranged to the corresponding pinacolones and the relative migratory powers of the phenyl, *o*-tolyl and *o*-anisyl groups have been determined.

Benzopinacol is oxidized by gaseous oxygen in acetic acid solution, either with or without the addition of sodium acetate, to benzophenone. The same oxidation is accomplished much more rapidly by a solution of iodine and sodium acetate in acetic acid.

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[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACOLOGY OF THE JOHNS HOPKINS  
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## CHEMICAL INVESTIGATION OF RAUWOLFIA CAFFRA. I. RAUWOLFINE

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The genus *Rauwolfia*, belonging to the natural order *Apocynaceae*, is widespread throughout the tropics and some forty-two species are known. Although most species are actively poisonous and many are reputed to possess medicinal value, information concerning their chemistry or pharmacology is extremely meager. There are occasional references in the literature wherein the investigators<sup>1</sup> lay claim to having obtained active crystalline bodies from some of the East Indian *Rauwolfia* but the investigations were limited in scope and the reports are vague. Accordingly, when a supply of the bark of a South African species of *Rauwolfia* became available, the present investigation was undertaken with the hope that some definite information would be forthcoming concerning the constituents of the bark responsible for the physiological activity and, furthermore, that if chemical individuals could be isolated, a study of their properties from a chemical and pharmacological viewpoint might prove of value.

The only available information concerning the South African *Rauwolfia* under investigation is contained in a report<sup>2</sup> from which the following facts are taken. An investigation was made of the nature of the active principle of the so-called "Quinine Tree" of the Transkei, the "*Umjela*" as it is known by the natives. This tree, now identified as *Rauwolfia caffra* (formerly as *Tabernaemontana ventricosa*, Hochst), grows in the Gxwaleni

<sup>1</sup> Wehmer, "Die Pflanzenstoffe," G. Fisher, Jena, 1931, Vol. ii, p. 981; Greschoff, *Ber.*, 23, 3543 (1890).

<sup>2</sup> Juritz, "Report of the Senior Analyst for the Year 1901," Cape Town, 1902, p. 58.

forest in the Nyanduli district and in the Mpami forest, Elliotdale, where specimens four feet in diameter and fifty feet in height are known to occur. The bark has a bitter taste and is reputed to have the well-known therapeutic properties of quinine. No alkaloids of the cinchona group were detected in the bark but needle-shaped crystals, which were thought to represent a new alkaloid, were isolated from a chloroform extract of the bark. The report concludes with a description of the properties of the crystals which, beyond the fact that they gave a crimson lake (Vitali's test), need not be included here as there is no evidence that an individual alkaloid had been isolated.

The present communication describes the progress made thus far in the investigation of this bark. Three crystalline basic substances have been obtained, one in sufficient quantity to permit of its more detailed study. The name "rauwolfine," derived from the generic name of the tree, is suggested for this base. The other two bases are designated Base A and Base B, respectively, until more is known concerning their chemistry and possible relationship to rauwolfine.

The properties of rauwolfine, such as its apparent instability in solution and in particular its insolubility in all solvents immiscible with water, leave much to be desired in the method used for its isolation from the bark. For the sake of brevity, therefore, only an outline of the operations performed is presented in this paper; a more detailed description is reserved for future communications when the information now available will no doubt have led to more efficient methods. Lastly, certain chemical aspects of rauwolfine, aside from its possible pharmacological importance, seem of sufficient interest to warrant the further efforts which are being made with a view to elucidating the chemical constitution of the base.

### Experimental

**Material.**—Through the kind coöperation of the Division of Plant Industry of the Department of Agriculture and the Forest Department of the Union of South Africa, a supply of the dry bark of *Rauwolfia caffra* Sonder (National Herbarium, Pretoria No. 3358) has been made available for this investigation. The material was collected in 1926, in the Mzwane Forest, Mtambalala Patrol, District Port of St. John's, Transkei, and after arrival in Baltimore was ground and stored in sealed containers.

**Extraction of the Bark.**—Ten kilograms of the finely ground dry bark<sup>3</sup> was completely extracted with hot petroleum ether (b. p. 30–60°) which yielded upon evaporation 100 g. of a light yellow waxy material that solidified in the ice chest and had a characteristic tea-like odor. This material gave negative tests for alkaloids and a portion was set aside for possible future investigation.

After petroleum ether extraction the ground bark was covered with 93% ethanol and boiled for five minutes. When cool the material was collected on a filter, transferred to a percolator and continuously extracted with fresh portions of cold 93%

<sup>3</sup> This opportunity is taken to thank the Directors and Laboratory Staff of Hynson, Westcott and Dunning for conducting the petroleum ether and ethanol extractions.

ethanol over a period of several weeks. When 240 liters of solvent had been used, the last percolates were still highly colored and gave copious precipitates with Mayer's reagent. The material was therefore exhausted further with 250 liters of 93% ethanol or until the percolate was but slightly colored and a test portion gave a negligible precipitate with Mayer's reagent. The last 250 liters of percolate, when concentrated under diminished pressure to 1.5 liters and placed in the ice chest for three weeks, deposited 3.5 g. of a crystalline substance which upon investigation proved to be *dl*-inosite melting at 223–224°.

The combined ethanol extracts of the bark were taken to virtual dryness under diminished pressure at 40°, which left 1040 g. of a brown varnish-like residue (representing 10% of the weight of the dry bark). The solid residue was covered with 1.5 liters of water and distilled with steam for five hours. An ethereal extract of the 3.5 liters of distillate yielded 6 g. of a sweet-smelling essential oil, together with 5 g. of *o*-hydroxybenzaldehyde, identified by means of its phenylhydrazone.

The flask containing the residue which had been subjected to steam distillation was allowed to stand overnight. The dark red supernatant liquid was then decanted from 700 g. of tarry residue and diluted with its own volume of water. After standing for several days the solution was filtered from 200 g. of an acid-insoluble resinous precipitate and concentrated to 2 liters under diminished pressure at 40°. After standing overnight in the ice chest the deep red solution, which appeared perfectly clear and was faintly acid to litmus, was repeatedly extracted with chloroform.

**Base A.**—The 3 liters of chloroform extract, after washing and drying and removal of the solvent, yielded 30 g. of a dark red viscous gum from which 0.015 g. of a crystalline alkaloid, designated Base A, was isolated after a series of operations involving precipitations with sodium carbonate and sodium hydroxide, together with extractions by means of dilute acid and ether. The base may be extracted from an alkaline solution with ether but is apparently insoluble in dry ether when crystalline. It is insoluble in water and petroleum ether but soluble in alcohol, chloroform and acetone and may be best crystallized from hot benzene and petroleum ether, from a mixture of which it separates in tufts of hair-like colorless needles melting at 294–295° (uncorr.) to a dark brown liquid. The Base A yields a yellow crystalline platinum chloride, a purple amorphous auric chloride and an amorphous picrate. It dissolves in concentrated sulfuric acid without color but produces a brilliant orange in concentrated nitric acid and a violet coloration fading to brown with Vitali's reagent. The base is precipitated from acid solution by sodium hydroxide, sodium carbonate and ammonium hydroxide in excess.

The main watery solution, together with the washings of the chloroform extraction, was reduced to 2 liters in volume and at the same time freed from dissolved chloroform by evaporation under diminished pressure at 40° and then precipitated with 150 g. of neutral lead acetate. After removal of the lead precipitate by filtering, the filtrate was delead with hydrogen sulfide, the excess acetic acid partially neutralized by addition of 20 g. of sodium carbonate, and the solution concentrated under diminished pressure at 40° to 2.5 liters. Preliminary experiments had indicated that a fractional precipitation by means of alkaline carbonate should be resorted to at this point in order to remove certain acid-soluble gums. Accordingly, 600 cc. of a 20% solution of sodium carbonate was slowly added, the supernatant liquid was decanted from the red-brown sticky precipitate which separated and the alkaline solution successively extracted with 3 liters of chloroform.

**Base B.**—The dark red chloroform extract, after washing with water and drying over anhydrous sodium sulfate, was concentrated to 50 cc. and allowed to evaporate at room temperature, which left 5 g. of a dark gum interspersed with crystals. Some of the crystals were picked out and examined and were obviously impure. They gave

copious precipitates with the usual alkaloidal reagents and were soluble in dilute acids; from the acid solutions the substance designated Base B could be recovered after addition of sodium carbonate in excess.

#### Rauwolfine

**Isolation.**—The alkaline solution, together with washings and some amorphous precipitate which appeared during the previous chloroform extraction, was made just acid to litmus with 100 g. of glacial acetic acid and the volume reduced to 1.5 liters under diminished pressure at 40°. To the clear liquid was slowly added a saturated solution of 150 g. of potassium carbonate. After standing for some time the solution had clarified and was decanted from the precipitated yellow gum and a further addition of 150 g. of potassium carbonate was made. This last increase in alkalinity threw down a greenish yellow amorphous, though granular, precipitate which was collected on the filter and washed with dilute potassium carbonate. It consisted for the most part of the base designated as rauwolfine. After two crystallizations from boiling water 10 g. of the base, representing 0.1% of the weight of the dry bark of *R. caffra*, was obtained as light buff-colored crystals. The base was further purified by three successive crystallizations from water, conversion into the crystalline chloride (see below) and regeneration of the free base, followed by two more crystallizations from water.

**Free Base.**—Rauwolfine crystallizes from water in small transparent, very light buff-colored tabular crystals (approximately 0.3 mm. in length). They appear to belong to the rhombic bipyramidal class of the orthorhombic system. The forms present, listed in the order of prominence, are the basal pinacoid, the side pinacoid, and the vertical rhombic prism. The general shape of the tablet is an elongated hexagon; two opposite angles are equal to 88°, the other four to 46° (these are the external angles). Optically the crystals show fairly high birefringence, the index of refraction is always greater than that of Canada balsam, the elongation is negative.<sup>4</sup> The base (dried at room temperature) has no definite melting point but when heated at a normal rate in an evacuated capillary tube it darkens at 200° and decomposes at 235–238°.

Rauwolfine in aqueous solution is strongly alkaline to litmus. It dissolves readily in all acids and solutions of its salts are neutral in reaction to litmus. The salts are not decomposed by sodium hydrogen carbonate. The free base is insoluble in sodium carbonate although soluble in sodium hydroxide, from a solution of which it is precipitated by carbon dioxide. Rauwolfine gives amorphous precipitates with all the usual alkaloidal reagents. The mercuric chloride precipitate is soluble in dilute sulfuric acid. The brown auric chloride partially reduces in the cold. The yellow picrate is decomposed on warming, likewise the orange iodo potassium iodide precipitate. The phosphotungstate is insoluble in water or alcohol. The tannate, soluble in dilute hydrochloric acid but reprecipitated by excess acid, is insoluble in ammonium hydroxide. A solution of the base in water decolorizes a neutral solution of potassium permanganate in the cold with the formation of a brown precipitate.

Rauwolfine gives two useful color reactions. A small quantity of the base dissolved in concentrated sulfuric acid produces a brilliant yellow solution. In time the color is gradually discharged; upon the addition of a drop of concentrated nitric acid to the colorless solution, a brilliant indigo-blue appears, passing rapidly through purple to a golden brown. The base dissolves in concentrated nitric acid to a deep wine-red fading to golden brown. The base does not give any marked color reaction with ferric chloride although a slight precipitate is produced in concentrated solution. Vitali's test is negative.

<sup>4</sup> From a cursory crystallographic examination made by Dr. J. D. H. Donnay of the Johns Hopkins University.

The behavior of rauwolfine with solvents is especially noteworthy. The crystalline base is only slightly soluble in cold water but readily so in hot (approximately one part in ten), and it is characterized by its insolubility in all the non-hydrolytic solvents tried, including pyridine and dioxane. The behavior of the base with alcohols seems exceptional—it is extremely soluble in absolute methanol but insoluble in absolute ethanol even on prolonged boiling. When 0.2 g. of the base was dissolved in 5 cc. of hot 90% ethanol and 15 cc. of absolute ethanol was added, the solution on partial cooling set to a jelly-like mass of crystals. When collected on the filter and washed with ether it formed a mat of hair-like colorless crystals weighing 0.050 g., a sample of which (dried at room temperature) decomposed at 227–229° when heated in an evacuated capillary tube. This substance turns green when heated in an air oven at 100° or when kept in an evacuated desiccator over phosphorus pentoxide. Attempts to analyze the substance gave unsatisfactory results but when it was recrystallized from water, rauwolfine of the usual crystal form and decomposition point resulted.

Although colorless specimens of rauwolfine and its salts have been obtained by precipitating them from freshly prepared solution, the application of heat necessary for recrystallization or prolonged exposure in solution always causes the appearance of some color. This color is no doubt due to products of decomposition and suggests that the base is relatively unstable in solution. An aqueous or alcoholic solution of the free base is olive-green and acidified solutions are pink; in either case the color deepens upon long standing.

*Anal.*<sup>5</sup> Calcd. (I) for  $C_{20}H_{26}O_8N_2$ : C, 70.17; H, 7.60. Calcd. (II) for  $C_{20}H_{26}O_8N_2 + 2.5$  moles of water: N, 7.23. Found (I) (dried at 100°): C, 69.54, 69.64; H, 7.44, 7.23. Found (II) (air-dried): N, 7.13, 7.07. Subs. (air-dried) 5.007, 4.997; loss at 100°, 0.584, 0.598. Calcd. for  $C_{20}H_{26}O_8N_2 + 2.5$  moles of water: 11.63. Found: 11.66, 11.97.

A Zeisel-Perkins determination of alkoxy groups gave completely negative results with rauwolfine and since the base proved to be insoluble in camphor, the determination of its molecular weight will have to be postponed until a suitable solvent is found. Preliminary attempts to prepare an acetyl derivative or a methiodide of the base were unsuccessful.

**Halogen Salts.**—Rauwolfine chloride was obtained in the anhydrous form by the following procedure. To 0.1 g. of rauwolfine in 2 cc. of cold absolute methanol, 10 cc. of absolute ethanol was added together with 0.05 cc. of concentrated hydrochloric acid. The solution was filtered and, after addition of 10 cc. of anhydrous ether, placed in the ice chest overnight. Next morning 0.06 g. of the salt in the form of faintly pink, pointed plates was collected on the filter and washed with ethanol and ether.

Rauwolfine chloride plus one molecule of water may be prepared by recrystallization of the anhydrous form from water or by dissolving the free base and making the solution just acid to congo paper with hydrochloric acid. The bromide and iodide were prepared in a corresponding manner; no attempt was made to obtain their anhydrous forms.

The halogen salts of rauwolfine are slightly soluble in cold water, soluble in hot; they are furthermore soluble in methanol in the cold but soluble only with difficulty in

<sup>5</sup> All the analyses in this communication were performed by Dr.-Ing. A. Schoeller of Berlin-Schmargendorf by the micro method and three to five milligrams of substance was usually taken for analysis. The notation "air-dried" signifies that the substance was dried to constant weight in a current of air at room temperature. The notation "dried at 100°" signifies that the substance was dried to constant weight at 100° in a high vacuum over phosphorus pentoxide.

TABLE I  
 HALOGEN SALTS OF RAUWOLFINE,  $C_{20}H_{26}O_2N_2$ -HALIDE

Salt	M. p. <sup>b</sup> (with dec.), °C.	Carbon, %			Hydrogen, %		
		Calcd.	66.80	66.70	Calcd.	7.07	7.00
Chloride anhydrous	300-303	66.57	66.80	66.70	6.93	7.07	7.00
Chloride + 1 mole H <sub>2</sub> O	297-300 (darkens 270)	66.57	66.16	66.26	6.93	7.29	7.09
Bromide + 1 mole H <sub>2</sub> O	250-253 (softens 245)	59.27	59.13	59.10	6.17	6.33	6.19
Iodide + 1 mole H <sub>2</sub> O	220-225 (dec. 300+)	53.11	53.41	53.40	5.53	5.88	5.89

  

Salt	Nitrogen, %			Halogen, %			Loss at 100°, %		
	Calcd.	7.66	7.59	Calcd.	9.84	9.90	Calcd.	0.40	0.20
Chloride anhydrous	7.76	7.66	7.59	9.84	10.08	9.90	0	0.40	0.20
Chloride + 1 mole H <sub>2</sub> O	7.39 <sup>a</sup>	7.47	7.36	9.84	10.08	10.33	4.75	4.10	4.32
Bromide + 1 mole H <sub>2</sub> O	6.62 <sup>a</sup>	6.50	6.42	19.73	19.39	19.63	4.25	4.02	3.99
Iodide + 1 mole H <sub>2</sub> O	5.95 <sup>a</sup>	5.88	5.85	28.08	28.24	28.46	3.83	4.52	3.86

<sup>a</sup> Calculated on "air-dried" basis, + 1 mole H<sub>2</sub>O. The other determinations were made on specimens dried at 100°.

<sup>b</sup> Normal rate of heating in an evacuated capillary tube.

boiling 95% ethanol. Their melting and decomposition points are not well defined and vary with the rate of heating.

Some difficulty has been experienced in measuring the optical activity of rauwolfine. However, Dr. R. T. Dillon of the Rockefeller Institute for Medical Research, who kindly made some measurements, obtained the approximate value  $[\alpha]_D^{20} + 29^\circ$  (in water) and  $[\alpha]_D^{20} + 45^\circ$  (in 95% ethanol) for the specific rotation of rauwolfine chloride.

**Empirical Formula.**—The empirical formulas derived from the analyses of rauwolfine and its salts are of necessity put forward only provisionally at this time. The formula  $C_{20}H_{26}O_2N_2Cl$  for the anhydrous form of rauwolfine chloride seems to agree satisfactorily with the analytical data. Until a molecular weight determination can be made, the supposition seems justified that such a naturally occurring alkaloid has two nitrogen atoms rather than four or more and consequently that the molecular formula of the salt is  $C_{20}H_{26}O_2N_2Cl$ . In the case of the free base of rauwolfine crystallized from water, the empirical formula  $C_{20}H_{26}O_3N_2 \cdot 2.5H_2O$  seems best in line with the analytical data. This would mean that the salts of rauwolfine are formed by loss of water although the possibility remains that the crystalline free base still retains some solvent of crystallization even when dried at 100° in a high vacuum over phosphorus pentoxide. No further conjecture need be made beyond the suggestion that rauwolfine may be an alkaloid of the quaternary ammonium base "type" and with the evidence at hand indicating such a possibility the nomenclature rauwolfine chloride rather than rauwolfine hydrochloride has been followed.

**Pharmacological Action.**—Rauwolfine or its salts, when injected into experimental animals, causes very definite pharmacological and toxico-

logical reactions.<sup>6</sup> The lethal dose for rats, cats, and rabbits is of the order of 35 mg. per kilogram of body weight. An intravenous or intraperitoneal injection of the drug causes, in the unanesthetized animal, restlessness and definite stimulation of the respiration, both as regards rate and amplitude, and later convulsions and cessation of the respiration occur and in some instances the heart continues to beat for several minutes.

Rauwolfine in doses of 3 mg. or more causes a definite curare-like action when injected into the lymph sacs of frogs, as evidenced by the paralysis of the motor nerve-endings in the skeletal muscles.

Rauwolfine when injected into anesthetized cats, dogs, and rabbits causes a fall in blood pressure and stimulation of the respiration—effects which can be repeatedly elicited and which also occur after a previous injection of atropine sulfate. From a pharmacological analysis it appears that the main site of action in mammals is on the brain centers, rather than in the peripheral structures. The drug has little or no effect on isolated smooth muscle.

Professor Reginald D. Manwell of the Department of Zoölogy, Syracuse University, has kindly carried out a series of tests with rauwolfine on canaries infected with *plasmodium elongatum* and *plasmodium praecox*, two species of avian malarial parasites. He found that the drug does not have antimalarial properties.

In conclusion the author desires to thank Professor John J. Abel and Dr. E. M. K. Geiling for their continued interest and encouragement, and Dr. James F. Couch for valuable criticism.

### Summary

A chemical investigation has been undertaken of *Rauwolfia caffra*, a South African tree, the bark of which is reputed to possess therapeutic value. The investigation has thus far yielded three crystalline bases, one of which, identified as a new alkaloid, has been named rauwolfine and provisionally assigned the formula  $C_{20}H_{26}O_3N_2 \cdot 2.5H_2O$ . Some of the properties of the alkaloid, including its pharmacological action, and the preparation and properties of its halogen salts are described.

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<sup>6</sup> This information is based upon a study of the pharmacological action of rauwolfine by Dr. E. M. K. Geiling, a detailed report of which will be published in the *Journal of Pharmacology and Experimental Therapeutics*.