marked so soon as they appeared, and the distance from the point of application measured.

Conventional R_f values were determined from system A; however, the ratio of distance moved by a sterol to distance moved by cholesterol was employed for simplicity in system B, in that the solvent front was frequently obscured. This ratio, the R_c value, is converted to R_f by multiplying by the R_f of cholesterol (0.63) in the Neher–Wettstein system. The mobilities in Table I are averages of many determinations. Spots IIA and IIB (system B) did not separate in every case, and an average of 0.14 was determined for the unresolved spot. Chromatography of 25 μg. quantities for 5–6 hours usually resolved the mixture. The R_f for the 7-hydroxycholesterols determined in system A is a value for the mixture.

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Rauwolfia Alkaloids. II. Isolation and Characterization of Two New Alkaloids from *Rauwolfia serpentina* Benth

BY NORBERT NEUSS, HAROLD E. BOAZ AND JAMES W. FORBES

RECEIVED FEBRUARY 1, 1954

Within the last year several authors have reported the isolation of new alkaloids from the Indian medicinal plant, *Rauwolfia serpentina* Benth.[1] In connection with our work on the structure of reserpine, the sedative principle of this plant, it was mentioned that chromatography of the oleoresin fraction yielded some new alkaloids in addition to reserpine.[2] The present note deals with these two new indole alkaloids. Both compounds are weak bases and isolated by chromatography of the oleoresin fraction on acid-washed alumina using benzene–chloroform mixtures as an eluant.

The first of the two alkaloids, tentatively called "alkaloid F," analyzed well for a $C_{21}H_{24}N_2O_3$ compound. This formulation was substantiated by the analysis of a hydrochloride. Comparison of the ultraviolet and infrared spectra (Fig. 2 and Fig. 1, A, B, respectively) of this alkaloid and tetrahydroalstonine (I)[3] indicates a close relationship of these two alkaloids. Their ultraviolet spectra are practically identical with the exception of a more intense shoulder at 248 mμ in the spectrum of tetrahydroalstonine. This difference was shown to be real since repeated recrystallizations of the sample did not alter the spectra.

The infrared spectrum of tetrahydroalstonine in chloroform differs in the following way from that of "alkaloid F": greater degree of hydrogen bonding of the NH group, broader and less symmetrical conjugated ester carbonyl band, 0.03 μ shorter wave length of the conjugated vinyl ether band and distinct differences in the longer wave length portion of the spectra. A series of spectra of both compounds at different concentrations in chloroform showed no evidence for intramolecular hydrogen bonding and no dependence of the breadth and shape of the carbonyl band upon concentration.

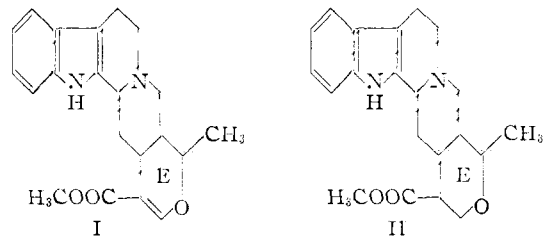
All of the observed differences between the spectra and the pK'_a values (see Experimental) of tetrahydroalstonine and "alkaloid F" could arise from differences in steric configuration of these two alkaloids. Comparison of several of the possible stereoisomers of tetrahydroalstonine, constructed with Fisher–Taylor–Hirschfelder models, shows considerable differences in the freedom of rotation of the carbomethoxy group and in the environment of the tertiary nitrogen.

The physical and analytical data of "alkaloid F" suggest the possibility of its identity with py-tetrahydroserpentine. This alkaloid was first prepared by Bader and Schwarz[4] by catalytic hydrogenation of serpentine in methanol at *p*H 10. The infrared and ultraviolet spectra were quite similar to those of our "alkaloid F." Lack of an authentic sample of py-tetrahydroserpentine did not permit the establishment of its identity with "alkaloid F." As soon as this can be done it is proposed to alter the formula of py-tetrahydroserpentine[4] (II) to I thus making it a steroisomer of tetrahydroalstonine.[5]

TABLE I

PHYSICAL PROPERTIES OF PY-TETRAHYDROSERPENTINE[4] AND "ALKALOID F"

Compound	M.p., °C.	$[\alpha]_D$	Infrared spectra, μ Ester CO	—C=C—O—	Solvent
Py-tetrahydroserpentine	249–250	−37 ± 6; −33 ± 6 (MeOH)	5.89	6.21	Methylene chloride
"Alkaloid F"	253–254	−44.6; −44.2 ($MeOH–CHCl_3$)	5.90	6.18	Chloroform
Py-tetrahydroserpentine HCl	276–277				
"Alkaloid F" HCl	264–265				



The second alkaloid, tentatively called "alkaloid A," analyzed well for a $C_{22}H_{26}N_2O_4$ compound. The formula was substantiated by analysis of a hydrochloride. The infrared and ultraviolet spectra of the alkaloid (Fig. 1 and Fig. 2, D) suggested a very close relationship to tetrahydroalstonine with a methoxy group at the 11-position of the heteroyohimbane ring system (III). Using the technique described in the previous paper,[2] we have recorded the composite infrared spectrum of tetrahydroalstonine and 2,3-dimethyl-6-methoxyindole at equimo-

(1) A. Stoll and A. Hofmann, *Helv. Chim. Acta*, **36**, 1143 (1953); K. Bodendorf and H. Eder, *Naturwissenschaften*, **40**, 342 (1953).

(2) N. Neuss, H. E. Boaz and J. W. Forbes, THIS JOURNAL, **75**, 4870 (1953); **76**, 2463 (1954).

(3) We thank Dr. R. C. Elderfield, of the University of Michigan, for the generous sample of tetrahydroalstonine. This structure for tetrahydroalstonine was first proposed by E. Schlittler, H. Schwarz and F. Bader, *Helv. Chim. Acta*, **35**, 271 (1952).

(4) F. Bader and H. Schwarz, *ibid.*, **35**, 1594 (1952).

(5) In a recent paper dealing with structure elucidation of tetrahydroalstonine, F. E. Bader, *ibid.*, **36**, 215 (1953), mentioned that there is a possibility that a double bond in the ring E of py-tetrahydroserpentine might have been overlooked.

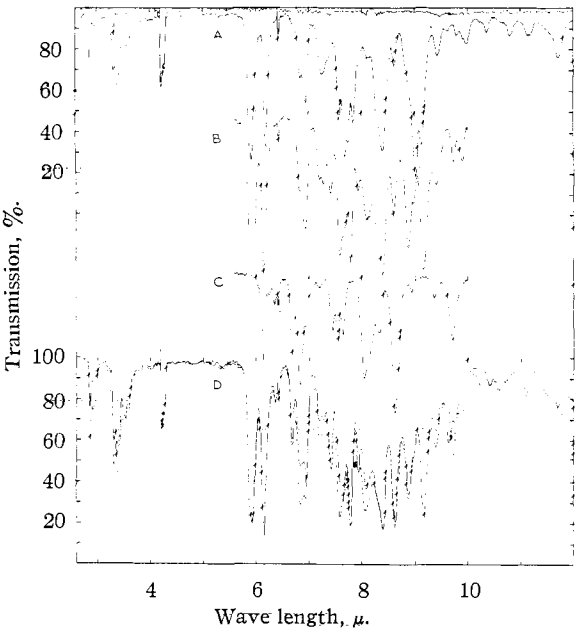
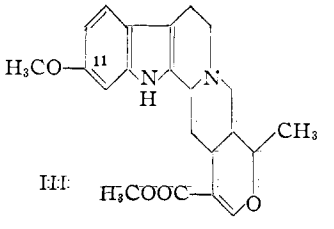


Fig. 1.—Infrared absorption spectra: A, alkaloid F, 4.8% solution and solvent blank; B, tetrahydroalstonine, 6.0% solution; C, 2,3-dimethyl-6-methoxyindole, 3.0% solution; D, solid line, alkaloid A, 6.5% solution. Dotted line, tetrahydroalstonine and 2,3-dimethyl-6-methoxyindole at 6.0% and 3.0%, respectively, in the same solution. All spectra were obtained from chloroform solutions in 0.100 mm. path with the Beckman IR-2T spectrophotometer. Solutes in spectra B, C and D are all at a concentration of 0.170 *M*.

lar concentration in chloroform. The identity of wave lengths and intensities of most of the corresponding bands in the two spectra not only proves the substitution at the 11-position by a methoxy group but also strongly suggests the same steric configuration in "alkaloid A" and tetrahydroalstonine. The similarity of the pK'_a values as well as optical rotations of the two molecules is in good agreement with such a formulation. The differences in intensities below 7.3 μ are due largely to the presence of the indole ring in both components of the above mixture, thus increasing the CH contribution and doubling the NH intensity. It is possible that "alkaloid A" is identical with Schlittler's compound 13141 for which he proposed the same structure III



III

without giving any physical data of the new alkaloid.[6]

ADDED IN PROOF.—Since the submission of this paper, several communications on isolation of new alkaloids from *Rauwolfia serpentina* have appeared in the literature.[7]

(6) E. Schlittler and H. Saner, Organic Symposium of the American Chemical Society, Ann Arbor, Mich., June 15–19, 1953.

(7) A. Popelak, *et al.*, *Naturwissenschaften*, **40**, 625 (1953); A. Hofmann, *Helv. Chim. Acta*, **37**, 314 (1954); M. W. Klohs, *et al.*, THIS JOURNAL, **76**, 1332 (1954); F. E. Bader, *et al.*, *ibid.*, **76**, 1695 (1954); F. L. Weisenborn, *et al.*, *Chemistry and Industry*, **73**, 375 (1954); E. Schlittler, *et al.*, *Experientia*, **10**, 133 (1954).

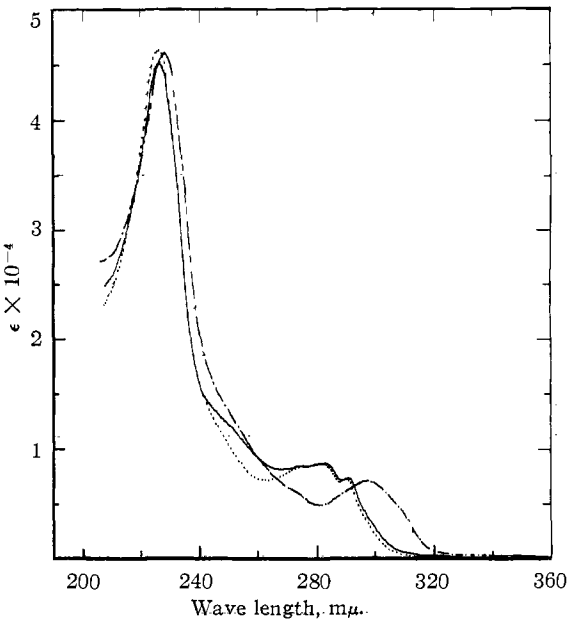


Fig. 2.—Ultraviolet absorption spectra of tetrahydroalstonine (———), alkaloid F (. . . . .) and alkaloid A (- · - · - ·). Spectra were taken in methanol solution using a Cary model 11 spectrophotometer; solutes are all at a concentration of 2.91 × 10^{-5} *M*.

The similarity of the physical properties of our "alkaloid A," substance I of Popelak[7] and reserpinine of Weisenborn[7] and Schlittler[7] indicates that these substances are identical.

Also we have received authentic samples of py-tetrahydroserpentine from Dr. Bader and ajmalicine, py-tetrahydroserpentine and py-tetrahydroserpentinol from Dr. Klohs. At this time we should like to express our appreciation for these samples.

Our "alkaloid F" was identical in every respect (X-ray patterns, infrared spectra, m.p. and mixed m.p.) with ajmalicine and py-tetrahydroserpentine of Klohs.[7] Py-tetrahydroserpentine of Bader[4] was identical (infrared spectra and X-ray patterns) with our sample prepared by catalytic hydrogenation of serpentine and crystallization from 80% methanol and was shown to be a hydrate of ajmalicine of Klohs. By heating to 110–120° it could be transformed into ajmalicine (identical X-ray patterns). By crystallizing "alkaloid F" from boiling 80% methanol, we obtained material identical with Bader's py-tetrahydroserpentine (identical X-ray patterns).

Therefore, our "alkaloid F," ajmalicine of Klohs and δ-yohimbine of Weisenborn[7] represent an anhydrous form of the py-tetrahydroserpentine of Bader.[4]

Acknowledgment.—We gratefully acknowledge invaluable assistance by the following: Messrs. W. L. Brown, G. M. Maciak and H. L. Hunter, elementary analyses and group determination; Mr. E. H. Stuart, isolation of the oleoresin fraction; Mrs. Barbara Kehm, technical assistance; Mr. D. O. Woolf and Mrs. H. Arndt, electrometric titrations and Miss M. Hofmann, infrared spectra.

Experimental[8]

Isolation and Characterization of Alkaloid A.—A solution of 10 g. of the oleoresin fraction in 300 ml. of thiophene-free benzene was chromatographed on a column (36 mm. in

(8) All melting points are uncorrected and were taken on a Fisher–Johns melting point apparatus. The substances were inserted at 150° unless otherwise mentioned. Electrometric titrations were determined in 66% dimethylformamide as a solvent.

diameter) of 300 g. of acid-washed alumina, Merck. A volume of 2 l. of benzene in 250-ml. fractions was used first and only traces of fat isolated. The elution was then continued with benzene–chloroform mixtures (5:1, 8:3 and 1:1 in fractions of 250 ml.) yielding a total of 1.91 g. of crude alkaloid A. After repeated crystallization from acetone–water 650 mg. of shiny, slightly yellowish plates was obtained, m.p. 243–244° dec., $[\alpha]^{25}D$ −131° (*c* 1.18 in $CHCl_3$). For analysis the sample was dried at 110° (0.05 mm.) for 8 hours.

Anal. Calcd. for $C_{22}H_{26}N_2O_4$: C, 69.09; H, 6.85; N, 7.33; OCH_3 (2), 16.23; mol. wt., 382.44. Found: C, 69.25; H, 6.80; N, 7.32; OCH_3 (2), 16.15; pK'_a 6.25 (electrometric titration); ϵ_{228} max 45,700, log ϵ 4.66; ϵ_{298} max 6,990, log ϵ 3.845; ϵ_{282} min 4,820, log ϵ 3.68; ultraviolet spectrum Fig. 2; infrared spectrum Fig. 1, D.

Alkaloid A Hydrochloride.—The hydrochloride was prepared by the usual procedure and recrystallized twice from methanol–ether, m.p. 237–238° dec. For analysis the sample was dried at 120° (0.5 mm.) for 4 hours.

Anal. Calcd. for $C_{22}H_{26}N_2O_4$·HCl: C, 63.07; H, 6.50; N, 6.69; Cl, 8.46. Found: C, 62.77; H, 6.60; N, 6.60; Cl, 8.01.

Isolation and Characterization of Alkaloid F.—After changing the solvent ratio successively from 1:1 benzene–chloroform to 1:2 and 1:3 and then finally to chloroform alone a total of 650 mg. of crude alkaloid was isolated. It was recrystallized twice from absolute methanol and afforded colorless, shiny prisms, m.p. 253–254° dec., $[\alpha]^{26}D$ −44.6, −44.2° (*c*, 0.408 in MeOH–$CHCl_3$ 2:3), $[\alpha]^{26}D$ −47.7° (*c*, 0.712 in pyridine). For analysis the sample was dried at 100° (0.3 mm.) for 6 hours.

Anal. Calcd. for $C_{21}H_{24}N_2O_3$: C, 71.57; H, 6.86; N, 7.95; mol. wt., 352.4. Found: C, 71.43, 71.61; H, 6.93, 6.81; N, 8.13; mol. wt., 348 ± 10 (electrometric titration pK'_a 6.46); ϵ_{226} max 46,000, log ϵ 4.662; ϵ_{283} max 8,540, log ϵ 3.93; ϵ_{291} max 7,230, log ϵ 3.86. Ultraviolet spectrum Fig. 2; infrared spectrum Fig. 1, A.

Alkaloid F Hydrochloride.—The hydrochloride of this alkaloid was prepared by the procedure of Schwarz and Bader[4] and melted at 264–265° dec. The sample for analysis was dried at 120° for 5 hours (0.5 mm.).

Anal. Calcd. for $C_{21}H_{24}N_2O_3$·HCl: C, 64.85; H, 6.48; Cl, 9.12. Found: C, 64.65; H, 6.57; Cl, 9.41.

Tetrahydroalstonine.—A slightly colored sample of the alkaloid was repeatedly recrystallized from absolute methanol and afforded colorless, shiny plates, m.p. 230–231° dec.; pK'_a 5.98 (electrometric titration), $[\alpha]^{26}D$ −100.7°, −102.3° (*c*, 0.418 in $CHCl_3$). Ultraviolet spectrum Fig. 2; infrared spectrum Fig. 1, B; ϵ_{226} max 45,200, log ϵ 4.655; ϵ_{283} max 8,540, log ϵ 3.93; ϵ_{291} max 7,230, log ϵ 3.86.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, HARPUR COLLEGE]

Salt Effects on the Indicator Acidity Function, H_0[1]

BY MARTIN A. PAUL

RECEIVED FEBRUARY 5, 1954

The effects of neutral salts on the indicator acidity function, H_0, have been measured by spectrophotometric means in 0.01, 0.1 and 1 *M* aqueous HCl solutions with the simple basic indicators, 2,4-dichloroaniline, *p*-nitroaniline, diphenylamine and *o*-nitroaniline. In 0.01 and 0.1 *M* HCl, the salt concentration effect is linear up to salt concentrations of at least 4 *M*, and is specific for the particular salt. The apparent acidity is increased by salts in the order, LiCl > NaCl > KCl, while it is decreased by tetramethylammonium bromide and tetraethylammonium bromide. Diphenylamine shows a larger salt effect than *p*-nitroaniline, attributable to its larger molecular size. The data support the conclusion that salt effects on the activity coefficients of the uncharged basic indicator molecules (which were measured independently by the solubility method) play a predominant role, with the implication that general salt effects may help to account also for the marked increase of $-H_0$ over log (H^+) with increasing concentration of strong acid itself, in the absence of added electrolyte.

Previous investigations[2a,b] in isolated instances have shown that neutral salts apparently have a marked effect on acidity as measured according to the method of Hammett and Deyrup[3] by means of uncharged basic indicators. Since there is evidence that certain acid-catalyzed reactions whose rates have been shown to follow the indicator acidity are likewise subject to large salt effects,[4-7] it seemed worthwhile to undertake a systematic investigation. Aside from its possible bearing on reaction kinetics, such an investigation promised to afford further insight into the nature of the indicator acidity function, H_0, itself, particularly in regard to its rapid increase with increasing electrolyte concentration in aqueous solutions of strong acids.

The effects of several neutral salts on H_0 were therefore measured in 0.01, 0.1 and 1 *M* aqueous HCl solutions, with the indicators 2,4-dichloroaniline, *p*-nitroaniline, diphenylamine and *o*-nitroaniline. These free bases show maximum light absorption at the respective wave lengths, 240, 380, 279 and 412 mμ, the cationic acid form in each case being transparent. In addition, the effects of salts on the activity coefficients of the free bases were determined for three of the indicators by the solubility method.

Experimental

The indicator acidity function, H_0, is defined by the equation[3]

$$H_0 = -\log (BH^+)/(B) + pK_a \qquad (1)$$

where K_a represents the acid ionization constant of BH^+, the cationic acid conjugate to the uncharged basic indicator, B. The concentration ratio, $(BH^+)/(B)$, was determined by means of light absorption of the basic form, B, as measured with a Beckman model DU spectrophotometer at a standard total concentration of the indicator in both forms. The indicator concentrations were approximately 5×10^{-5} *M* for diphenylamine, 8×10^{-5} *M* for 2,4-dichloroaniline and for *p*-nitroaniline, and 2×10^{-4} *M* for *o*-nitroaniline. Beer's law was satisfied within experimental precision at these concentrations. Corrections were made where necessary for light absorption by the salt.

(1) Paper presented at the Chicago Meeting of the American Chemical Society, September, 1953.

(2) (a) L. P. Hammett and M. A. Paul, THIS JOURNAL, **56**, 827 (1934); (b) G. Harbottle, *ibid.*, **73**, 4024 (1951).

(3) L. P. Hammett and A. J. Deyrup, *ibid.*, **54**, 2721 (1932).

(4) Sucrose inversion, L. P. Hammett and M. A. Paul, *ibid.*, **56**, 830 (1934).

(5) Hydrolysis of β-propiolactone, F. A. Long and M. Purchase, *ibid.*, **72**, 3267 (1950).

(6) Trioxane decomposition, M. A. Paul, *ibid.*, **74**, 141 (1952).

(7) Methylal hydrolysis, F. A. Long and D. McIntyre, *ibid.*, **76**, 3240, 3243 (1954).