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Alkaloids of *Rauwolfia heterophylla*[1]

BY F. A. HOCHSTEIN, KOTARO MURAI AND W. H. BOEGEMANN

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Seven alkaloids, one of them previously unreported, have been isolated from *Rauwolfia heterophylla*. They are reserpine, ajmalicine, rauwolscine, yohimbine, ajmaline, serpentine and heterophyllin. Heterophyllin, a new alkaloid with the empirical formula $C_{22}H_{26}N_2O_4$, appears to be related in structure to ajmalicine.

The increasing medicinal use of reserpine, an alkaloid first isolated from the Indian plant, *Rauwolfia serpentina*,[2] has led to the examination of other *Rauwolfia* species. *Rauwolfia heterophylla* Roem. et Schult., a small shrub occurring in Central America, has been examined by earlier workers,[3,4] who isolated in pure form the alkaloids reserpine, ajmaline and serpentine. We have confirmed the presence of these substances in *R. heterophylla*, and have isolated four other alkaloids previously unreported in this plant. Of these, rauwolscine, yohimbine and ajmalicine have been isolated from other *Rauwolfia* species[5]; a fourth alkaloid, heterophyllin, has not been described previously.[6]

A deliberate search was made for certain other alkaloids reported from *Rauwolfia* species, rescinnamine,[7a] sarpagine,[7b] narcotine,[3,8] thebaine[9] and papaverine.[9] These occur, if at all, in concentrations of less than 0.001%.

There is evidence, based on paper chromatography and limited countercurrent distribution studies, that *R. heterophylla* contains at least twelve alkaloids. Paper chromatographic examination of crude fractions isolated from *R. heterophylla* branches suggest that the alkaloid distribution is qualitatively though not quantitatively similar to that of root. Leaf extracts contained little or no reserpine and paper chromatography indicated the presence of alkaloids not found in root extracts.

Preliminary extraction of *R. heterophylla* root of Mexican origin, obtained from a commercial source, was effected with anhydrous methanol. The concentrated extracts then were separated into three major fractions. The first fraction, a mixture of weak bases containing reserpine, ajmalicine and heterophyllin, is characterized by the solubility of the crude acetates in chloroform, while the second fraction, including ajmaline, rauwolscine and yohimbine, contained the remaining weak bases. A third strongly basic fraction yielded serpentine. Further fractionation utilized a variety of conventional procedures including countercurrent distribution, column chromatography, and direct crystallization of the free alkaloid bases or their salts.

Countercurrent distribution studies on the crude alkaloid fractions permitted the ready separation of the weak bases from the stronger bases. Preliminary studies indicated that further resolutions of either class were possible, but this technique was not used extensively for the actual isolation due to the limited capacities of our equipment and the small amounts of alkaloids occurring in the crude extracts. Paper chromatographic examination of various alkaloid fractions was an invaluable aid which permitted certain alkaloids to be followed with relative ease through the various separations.

The new alkaloid, heterophyllin,[9a] is a weak base, m.p. 186–187°, $[\alpha]^{25}D$ −89°. Analyses of heterophyllin and of several of its salts indicate an empirical formula $C_{22}H_{26}N_2O_4$. The new substance contains two methoxyl groups and no N-methyl groups. The ultraviolet absorption spectrum with peaks at 228 and 280 mμ is similar in a broad sense to that of the indole alkaloids ajmalicine, reserpinine and reserpiline.[10] The infrared spectrum (Fig. 1) is very similar to that of ajmalicine in the 3–7 μ region.[11]

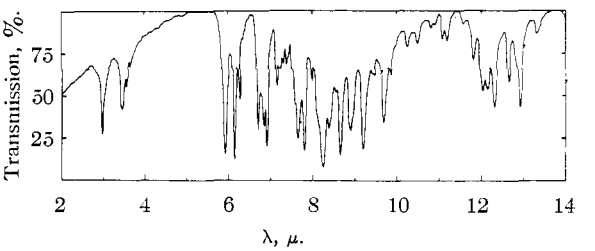

Fig. 1.—Infrared absorption spectrum of heterophyllin, in a potassium bromide pellet.

(1) Dr. L. Nickell has called our attention to the fact that *R. hirsuta* Jacq. is a preferred name for this plant species. See R. E. Woodson, Jr., *North American Flora*, **29**, (2), 134 (1938). We have continued to use the name *R. heterophylla*, in view of the earlier chemical publications using this designation.

(2) J. M. Mueller, E. Schlittler and H. J. Bein, *Experientia*, **8**, 338 (1952).

(3) C. Djerassi, M. Gorman, A. L. Nussbaum and J. Reynoso, THIS JOURNAL, **76**, 4463 (1954).

(4) M. M. Janot, R. Goutarel and A. LeHir, *Compt. rend.*, **238**, 720 (1954).

(5) A recent review of this general subject was published by E. Schlittler, J. A. Schneider and A. J. Plummer, *Angew. Chem.*, **66**, 386 (1954).

(6) K. Mezey and B. Uribe, *Ann. Soc. Biol. Bogota*, **6**, [3] 127 (1954); *Biol. Abstracts*, **29**, 169 (1955), recently have reported that *R. hirsuta* of Columbian origin contains alstonine and an alkaloid, m.p. 230–232°, resembling rauwolscine.

(7) (a) M. W. Klohs, M. D. Draper and F. Keller, THIS JOURNAL, **76**, 2843 (1954); (b) A. Stoll and A. Hofmann, *Helv. Chim. Acta*, **36**, 1143 (1953).

(8) Narcotine was found as a contaminant of an early sample of *R. heterophylla* studied by Djerassi (ref. 3). We examined our material for it, as well as for thebaine and papaverine which have been reported in *R. serpentina*.

(9) A. Hoffman, *Helv. Chim. Acta*, **37**, 849 (1954).

(9a) ADDED IN PROOF.—Since this paper was accepted for publication, "heterophyllin" has been unequivocally identified as aricin. We are indebted to Dr. M. Janot and Dr. Raymond-Hamet for authentic samples of aricin and to Dr. C. Djerassi for calling this possibility to our attention. A. Stoll, A. Hoffmann and R. Brunner (*Helv. Chim. Acta*, **38**, 270 (1955)) have recently reported the isolation of aricin from leaves of *R. canescens*, and have shown it to be 5-methoxyajmalicine. Dr. M. Janot, in a private communication, concurs with this structure assignment. The name "heterophyllin" should therefore be dropped in favor of aricin.

(10) M. W. Klohs, M. D. Draper, F. Keller and W. Malesh, *Chemistry & Industry*, 1264 (1954).

(11) M. W. Klohs, M. D. Draper, F. Keller, W. Malesh and F. J. Petracek, THIS JOURNAL, **76**, 1332 (1954).

It shows, for example, a single sharp peak at 3.0 μ, attributed to N–H absorption, and the characteristic pair at 5.95 and 6.17 μ associated with the α,β-unsaturated ester-enol ether function. This evidence suggests that heterophyllin is an isomer of reserpinine, and another member of the indole series with an oxygenated E ring.

Heterophyllin does not share the hypotensive or sedative properties of reserpine.

Experimental

All melting points were determined in capillary tubes, and are corrected. Rotations were determined at 1% concentration. All yields are based on the weight of root taken.

Extraction of *Rauwolfia heterophylla* Root.—Five hundred grams of dry *R. heterophylla* root was ground in a hammer-type mill to pass a 15-mesh screen. The ground product, containing 5% moisture, was stirred overnight at reflux temperature with 3 liters of methanol, filtered, and the residue washed with 1 liter of methanol. The hot extraction was repeated twice with 2-liter portions of solvent. The combined methanol extracts were concentrated *in vacuo* to 300 ml. and held at 0° overnight. Nine grams of a crude crystalline precipitate was separated, and recrystallized from aqueous methanol to yield 6.5 g. (1.3%) of pure sucrose, m.p. 184.5–185.5°, $[\alpha]^{25}D$, +68° (water). A mixed melting point with authentic sucrose was not depressed.

The sucrose filtrate was concentrated to dryness *in vacuo*, to yield 40 g. of tarry solids. This solid was dissolved in 100 ml. of methanol and mixed with 200 ml. of 5% aqueous acetic acid. The turbid suspension was extracted with two 250-ml. portions of hexane to remove 3.1 g. of a dark viscous oil, virtually free of alkaloids. The acid solution was cooled to 5°, adjusted to *p*H 10 and the amorphous precipitate filtered. The dried crude alkaloid separated in this way (8.5 g.) was combined with an additional 0.5 g. recovered by chloroform extraction of the filtrate; total weight, 9.0 g. (1.8%).

Preliminary Fractionation of the Crude Alkaloids.—The total crude alkaloids were dissolved in 100 ml. of chloroform, and extracted with four 100-ml. portions of 5% aqueous acetic acid. The chloroform solution then was washed with 5% ammonium hydroxide, and concentrated to dryness giving 2 g. of a crude fraction A which subsequently yielded reserpine, ajmalicine and heterophyllin.

The acetic acid extract from fraction A was cooled, adjusted to *p*H 7.5, and the amorphous alkaloid precipitate filtered and dried; weight 3.1 g. This fraction B subsequently yielded rauwolscine, yohimbine and ajmaline.

The filtrate from fraction B was adjusted to *p*H 10.5, and extracted with chloroform. Concentration of the extract gave about 2 g. of a dark orange red glass, fraction C, which subsequently yielded serpentine. Tars which formed during the separations were discarded. Further isolations were carried out on these fractions, and on fractions obtained in a similar manner from a 10-kg. pilot plant extraction.

Reserpine.—Fraction A of the preliminary isolation was ground in a mortar with three 20-ml. portions of ether, and filtered from 1.0 g. of ether-insoluble powder, which was rich in reserpine. The ether-insoluble powder was dissolved in 10 ml. of warm anhydrous ethanol, and filtered from 0.2 g. of undissolved tars. After standing in a refrigerator overnight, 0.175 g. of crude crystalline reserpine was separated by filtration. The crude reserpine was dissolved in 20 ml. of benzene, filtered and concentrated to about 0.5 ml. *in vacuo*. On addition of 5 ml. of methanol, the glass dissolved, and promptly precipitated 0.150 g. of virtually pure reserpine (0.03%). After one similar recrystallization, the product had $[\alpha]^{25}D$ −120° (chloroform) and melted at 263–265° dec. when placed in a bath at 250°, temperature rising 3° per minute. The melting point in an evacuated tube, under similar heating conditions, was 284–286° and is less dependent on rate of heating. The ultraviolet and infrared absorption spectra, and the behavior on two paper chromatographic systems were identical to those of an authentic sample of reserpine.

Ajmalicine and Heterophyllin.—The combined ether washes and ethanol mother liquors from the reserpine isolation were concentrated to dryness, and combined with fifteen grams of similar fractions representing a total of 5 kg. of root. This material was dissolved as completely as possible in 100 ml. of hot benzene, and filtered from several grams of tar. Paper chromatography showed this material to contain traces of reserpine, and appreciable amounts of three other alkaloids which moved somewhat faster on paper chromatograms. The solution was chromographed on a 45 × 200 mm. column of acid-washed alumina,[12] and eluted first with benzene, then with benzene containing increasing quantities (1, 2, 5, 10%) of methanol. The progress was followed by paper chromatography of the arbitrarily selected cuts, which averaged 100–200 ml. in volume. Resolution was not complete since pure fractions were not obtained in any single cut.

The first fraction, virtually unabsorbed by the column, yielded 0.6 g. of colorless non-alkaloidal oil. A second fraction, eluted with pure benzene, yielded 0.5 g. of crude alkaloid rich in heterophyllin. A third fraction, eluted with benzene containing 1% methanol, contained 1.60 g. of crude ajmalicine and traces of reserpine and heterophyllin. Further elution, finally with pure methanol, yielded additional traces of reserpine, and 12 g. of total solids, from which no further crystalline compounds were isolated.

The ajmalicine-rich fractions were dissolved in 5 ml. of hot methanol and filtered after several hours from a few mg. of crystalline reserpine. The solution then was diluted with water until turbid. Ajmalicine, 0.250 g. (0.005%) crystallized on standing overnight. Recrystallization from a mixture of benzene and methanol yielded 0.21 g. of pure ajmalicine, m.p. 262.5–263° (*in vacuo*),[11] $[\alpha]^{25}D$ −59° (*c* 0.5 in chloroform). The infrared spectrum and ultraviolet spectrum also were identical to those described by Klohs.[11] A portion was converted to the hydrochloride salt, m.p. 270–280° dec. open tube, 295–296° dec. *in vacuo*.

The heterophyllin-rich fraction, when dissolved in 3 ml. of methanol and seeded with ajmalicine, yielded a small additional amount of ajmalicine. The filtered solution was acidified with 0.25 ml. of glacial acetic acid. The dark solution precipitated 0.28 g. of dense colorless crystals of heterophyllin diacetate, which was purified by recrystallization from hot methanol–acetic acid, then from benzene; yield 0.21 g. (0.0042%). The product was dried *in vacuo* at 56° for 0.5 hour for analysis; m.p. 152–155° dec., $[\alpha]^{25}D$ −69° (chloroform).

Anal. Calcd. for $C_{22}H_{26}N_2O_4 \cdot 2C_2H_4O_2$: C, 62.14; H, 6.82; N, 5.57; methoxyl (2), 12.35; acetyl (2), 17.13. Found: C, 62.32; H, 6.84; N, 5.66; methoxyl, 13.01; acetyl, 16.72.

Additional quantities of heterophyllin diacetate subsequently were crystallized directly from the reserpine mother liquors of a pilot plant preparation. Closed bottles of the diacetate develop a strong odor of acetic acid on standing.

Heterophyllin base, prepared by washing a chloroform solution of the acetate with 5% ammonium hydroxide, was purified to constant melting point by crystallization twice from methanol and twice from isopropyl alcohol. The heavy needles (prisms) could be sublimed slowly at 180° (0.01 mm.) with no observed change in properties; m.p. 186.5–187.5° *in vacuo* or open tube, $[\alpha]^{25}D$ −89° (chloroform), −66° (pyridine).

Anal. Calcd. for $C_{22}H_{26}N_2O_4$: C, 69.09; H, 6.85; N, 7.33; methoxyl (2), 16.24; C-methyl (1), 3.93; mol. wt., 382.45. Found: C, 68.89; H, 6.75; N, 7.33; methoxyl, 16.39; N-methyl, 0.0; C-methyl, 4.47.

Titration in 1:1 dimethylformamide–water showed an apparent pK_a 6.8, equiv. wt. 378. The ultraviolet absorption spectrum in methanol shows peaks at 228 mμ, log ϵ 4.52 and at 280 mμ, log ϵ 3.98 and a minimum at 265 mμ, log ϵ 3.85. The infrared absorption spectrum (Fig. 1) bears a general similarity to that of ajmalicine.

Heterophyllin gives a red-brown color in sulfuric acid–formaldehyde. With ferric chloride in sulfuric acid it yields a yellow color turning to red-brown. Concentrated nitric acid gives an orange color.

Heterophyllin nitrate was prepared by the addition of nitric acid to a hot aqueous methanolic solution of the diacetate. The product was recrystallized from 85% methanol, and had a m.p. 258.1–259.5° dec. in open tube or *in vacuo*, when placed in a bath at 250°, temperature rising at 2–3° per minute. The compound was dried to constant weight *in vacuo* at 110°.

(12) D. A. Prins and C. W. Shoppee, *J. Chem. Soc.*, 498 (1946).

Anal. Calcd. for $C_{22}H_{26}N_2O_4 \cdot HNO_3$: C, 59.31; H, 6.11; N, 9.43. Found: C, 59.15; H, 5.97; N, 9.63.

Heterophyllin picrate was prepared by dissolving 0.10 g. of heterophyllin and 0.1 g. of picric acid in 1-ml. portions of hot ethanol, and mixing. The crystalline precipitate was recrystallized from 15 ml. of hot ethanol as beautiful anisotropic squares and cubes. This material, 0.07 g., was dried to constant weight at 80° (0.2 mm.) for analysis; m.p. 213–215° dec. open tube or *in vacuo*, when placed in a bath at 200°, temperature rising at 3° per minute.

Anal. Calcd. for $C_{22}H_{26}N_2O_4 \cdot C_6H_3N_3O_7$: C, 54.99; H, 4.78; N, 11.45; methoxyl (2), 10.10. Found: C, 55.29; H, 5.02; N, 11.71; methoxyl, 9.91.

Heterophyllin hydrochloride prepared by the addition of 0.5 ml. of 2 *N* hydrochloric acid to 150 mg. of heterophyllin in 2 ml. of ethanol did not crystallize immediately from this solvent. The solvent was removed *in vacuo* to eliminate excess acid, and the damp residue crystallized from hot nitromethane as long sturdy needles. After drying *in vacuo* at 80°, the material melted at 239–241° dec. when placed in a bath at 215°, temperature rising at 3° per minute.

Anal. Calcd. for $C_{22}H_{26}N_2O_4 \cdot HCl \cdot {}^1/_2H_2O$: C, 61.75; H, 6.59; N, 6.55. Found: C, 62.00; H, 6.58; N, 6.40.

A crystalline oxalate, small needles, m.p. 233–235° dec., and a tartrate, m.p. 197–200° dec., also were prepared from ethanol solution.

Rauwolscine, Yohimbine and Ajmaline.—Twenty-one grams of fraction B, representing 5 kg. of root, was dissolved in 250 ml. of chloroform, and washed with *p*H 8 phosphate buffer. The buffer wash removed 1.0 g. of alkaloidal solids which yielded 0.3 g. of crystalline serpentine on crystallization from ethanol. Tar, 2 g., which formed during the extraction, was discarded.

The chloroform solution then was extracted with four 100-ml. portions of 5% oxalic acid, and the oxalate solution held at 0° for 2 hours. The crystalline precipitate, 1.9 g., of rauwolscine and yohimbine oxalates which separated, was held and combined with a later fraction of similar alkaloid composition. The filtrate was adjusted to *p*H 10, and extracted into chloroform; weight 15 g. The chloroform solution, 100 ml., was washed with 0.2 *M* phosphate–citrate buffer at *p*H 6.6. Additional citric acid was added to maintain the *p*H at this level. Tars were discarded. The chloroform–buffer solutions were subjected to a 25-plate countercurrent distribution, and all tubes finally made basic with excess ammonia. The alkaloids obtained by concentrating the chloroform layers yielded a weight distribution curve with peaks at tube 0 (chloroform-soluble) and at tube 24 (buffer-soluble). Paper chromatographic examination showed discrete alkaloidal peaks at tubes 2–4 (rauwolscine and yohimbine), 8, 12 and 14–21 (ajmaline). Tubes 2–4, which contained 1.8 g. of crude alkaloids similar in composition to the crystalline oxalate isolated earlier, were converted to the oxalate, and yielded an additional 0.5 g. of crystalline oxalate salt. The combined oxalate salts were purified by digestion in 30 ml. of boiling anhydrous ethanol for 30 minutes, which converted the original very fine needles to a denser needle type. After drying at 70° for 1 hour this oxalate melted sharply at 265–266° dec. Paper chromatography indicated it to be a mixture of at least two alkaloids.

The oxalate was suspended in chloroform, dissolved by shaking with 5% ammonium hydroxide, and the chloroform solution of base concentrated to dryness. The base, crystallized three times from benzene, m.p. 218.5–220°, still showed two components by paper chromatography. Four-tenths gram of this base in 1 ml. of methanol was mixed with 0.35 g. of picric acid in 3 ml. of methanol and 0.5 ml. of water was added. The crystalline precipitate, 0.29 g., m.p. 191–195° dec., was chromatographically homogeneous. Reconversion to the base, and crystallization from benzene yielded pure rauwolscine, m.p. 233–235° dec., mixed m.p. with an authentic sample not depressed. The ultraviolet and infrared spectra were identical to those of an authentic sample; yield 0.015%.

The mother liquors from the preparation of the rauwolscine picrate were converted to the base, and crystallized first from benzene–ligroin, then from methanol, to yield pure yohimbine, m.p. 219–221°, mixed melting point with an authentic sample not depressed. The infrared spectrum was identical to that of authentic yohimbine, and the behavior on two paper chromatographic systems also was identical; yield, based on root, 0.010%. A semi-quantitative examination of the infrared spectrum of the mixed rauwolscine–yohimbine bases showed that they were present in substantially equal amounts, equivalent to about 0.02% of each in the root.

Tubes 14–22 from the distribution, containing a total of 1.12 g. of solids, were crystallized from methanol to yield 0.5 g. of crude crystalline ajmaline (0.01%). After one recrystallization from methanol, this product softened and swelled at 150°, melted at 157–160°. The infrared absorption spectrum and behavior on paper chromatography were identical to those of an authentic specimen.

Tubes 8 and 12 of the distribution contained less than 40 mg. of crude alkaloid mixture in each. No pure compounds were isolated.

Serpentine.—Fraction C of the preliminary fractionation, 2 g., was dissolved in 5 ml. of anhydrous ethanol. Crystalline serpentine, 0.85 g., separated overnight. Recrystallization from hot ethanol yielded 0.75 g. of pure product (0.15%), m.p. 153–154° dec., mixed melting point with an authentic sample not depressed. A portion was converted to the hydrochloride, m.p. 247–249° dec., $[\alpha]^{25}D$ +181° (water).

Other Alkaloids.—A 500-g. portion of ground dry *R. heterophylla* root was processed by Stoll's[7] procedure for the isolation of sarpagine. The "sarpagine" fraction contained 20 mg. of amorphous alkaloids, which did not crystallize on seeding with sarpagine. Paper chromatography indicated less than 5 mg. of sarpagine. This alkaloid, if present at all, must constitute less than 0.001% of the whole root.

Careful paper chromatography of reserpine mother liquors did not reveal the presence of any rescinnamine. Pure rescinnamine can be separated from reserpine, although with some difficulty, on our paper chromatographic systems.

Narcotine, thebaine and papaverine were not detected in any fraction by paper chromatography. This procedure should detect the presence of these alkaloid unless they occur in concentrations of less than 0.001% in dry root.

R. heterophylla **Branches and Leaves.**—Methanol extracts of ground branches and ground leaves were separated into two portions corresponding to fractions A, and fraction B + C, by the procedures described above for root extracts. Paper chromatographic examination of the branch extracts indicated that the qualitative distribution of alkaloids was substantially identical to that found in root. It was noted that the reserpine content of branches was lower than that of root. Paper chromatograms of the leaf extracts showed little or no reserpine, and several spots not observed in root extracts.

Countercurrent Distribution.—Exploratory 49-plate Craig countercurrent distributions performed on concentrates of root extract showed that the stronger bases (*e.g.*, serpentine) remained in the aqueous phase while the weaker bases were extracted into the organic phase. Results of typical runs are summarized in Table I.

TABLE I

DISTRIBUTION COEFFICIENTS OF ALKALOIDS

Alkaloid	System[a] A	B	C
Yohimbine	9	0.01	1.5
Rauwolscine	9	.01	0.7
Reserpine	9	.01	8
Ajmalicine	11	.01	8
Heterophyllin	20	.01	8
Serpentine	0.01	11–13	*ca.* 7
Ajmaline	0.01	11–13	*ca.* 1

[a] System A = benzene *vs.* 0.05 *M* *p*H 7.0 phosphate buffer; B = 50% methanol–water *vs.* chloroform (ref. 13); C = *n*-butyl alcohol *vs.* 15% aqueous acetic acid.

The methanol extract of *R. heterophylla* root was separated into two fractions I (weak bases) and II (strong bases) by a 10-plate Craig countercurrent distribution using the

(13) L. Dorfman, A. Furlenmeier, C. F. Huebner, R. Lucas, H. B. MacPhillamy, J. M. Mueller, E. Schlittler, R. Schwyzer and A. F. St. André, *Helv. Chim. Acta*, **37**, 59 (1954).

50% methanol–water–chloroform system. Paper chromatography of fraction I using the benzene–cyclohexane–formamide system indicated the presence of yohimbine (R_f 0.03), rauwolscine (R_f 0.08), reserpine (R_f 0.41), ajmalicine (R_f 0.67), heterophyllin (R_f 0.83) and an unidentified substance (R_f 0.52). Paper chromatography of fraction II using the water–acetic acid vapor system indicated the presence of serpentine (R_f 0.13), ajmaline (R_f 0.60) and four other materials (R_f 0.26, 0.40, 0.50, 0.78). Studies are in progress on these as yet uncharacterized substances.

Trial 15-plate Craig countercurrent distributions using the *n*-butyl alcohol–15% acetic acid system led to the separation of ajmaline from serpentine, and the group reserpine–ajmalicine–heterophyllin from the group yohimbine–rauwolscine (see system C, Table I). These groups could then be resolved conveniently into their components by the use of column chromatography, and subsequent crystallization of the alkaloids as the free bases or as salts.

Paper Chromatography.—Systems used for the paper chromatography of the alkaloids were: (1) water (developer) in water–acetic acid atmosphere, (2) benzene–cyclohexane (1:1) on formamide-impregnated paper,[14] (3) benzene–chloroform (1:1) on formamide-impregnated paper. Whatman #1 paper was used. The alkaloids were located on the oven-dried papers (90°, 1 hour) by their ultraviolet absorbance or fluorescence.

It was necessary to use known alkaloids as control references since R_f values varied with the amount of material applied and temperature. R_f values for the alkaloids cited in this paper are summarized in Table II.

Acknowledgments.—We should like to express our thanks to Dr. C. Djerassi for much helpful information and advice, and for the specimens of branches and leaves used in this investigation.

(14) A. Zaffaroni, *et al.*, *Science*, **111**, 6 (1950). Also O. Schindler and T. Reichstein, *Helv. Chim. Acta*, **34**, 108 (1951).

TABLE II

R_f VALUES FOR ALKALOIDS

Alkaloids	Systems[a] 1	2	3
Yohimbine	0.45	0.03	0.40
Rauwolscine	.55	.08	.62
Reserpine	.20	.41	.9
Ajmalicine	.18	.67	.9
Heterophyllin	.27	.83	.9
Serpentine	.13	.0	.0
Ajmaline	.60	.0	.15
Sarpagine	.40	.0	.0
Rescinnamine	0.0–0.18	.38	.9
Papaverine	0.52	.70	.9
Narcotine	.56	.82	.9
Thebaine	.50	.22	.9

[a] The systems are those referred to in the Discussion.

We are grateful too to Drs. A. Chatterjee, C. Djerassi, M. Klohs and E. Schlittler for specimens of authentic alkaloids. Drs. S. Y. P'An and D. Hutcheon kindly provided the pharmacological information. Messrs. G. Hess and T. Toolan and staffs provided all analyses and physical measurements. Messrs. A. Timreck and P. Guercio graciously provided the crude extracts of plant material identified by Dr. L. Nickell. We are grateful to Dr. A. Bavley for his interest and suggestions. We thank Mrs. R. Paradies for her very capable technical assistance.

BROOKLYN 6, N. Y.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, THE FLORIDA STATE UNIVERSITY]

Furano(3,2-c)pyridines[1]

BY WERNER HERZ AND STANLEY TOCKER

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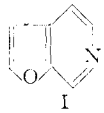
Isoquinoline ring syntheses were investigated in the furan series. A method for applying the Bischler–Napieralski reaction to derivatives of 2-(2-furyl)-ethylamine was devised and the reactions of the products studied. The Pomeranz–Fritsch, Pictet–Spengler and Pictet–Gams reaction could not be carried out successfully.

Replacement of the benzene moiety of isoquinoline by a furan nucleus gives rise to the isosteres shown below. It was of interest to develop syntheses of compounds of this type for the purpose of correlating their physiological properties with those of well-studied benzene analogs.



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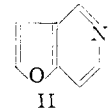

II

The synthesis of furan analogs of isoquinolines from furan derivatives seemed quite formidable in view of the high acid concentration required for the usual isoquinoline ring syntheses[2] which are based on imines, amides or iminoacetals. In fact furan compounds often resinify or undergo ring cleavage[3] under conditions far milder than those employed in cyclizations of this type.[2]

As expected, attempts at cyclization of amides prepared from 2-(2-furyl)-ethylamine by standard methods[2] gave only intractable resins resembling the tars obtained on treating furans with acid. Many experiments indicated that the concentration of acidic material at any given time was critical. A method of cyclization was therefore developed which takes advantage of the fact that the phosphate salt of the cyclized compound is insoluble in toluene and far more stable to carbon–oxygen cleavage than the uncyclized amide. A dilute toluene solution of phosphorus oxychloride was added slowly to a refluxing dilute solution of the amide in toluene. The desired salt was continually precipitated after a critical phosphorus oxychloride concentration (as low as 290 ml. of 0.012 *M* $POCl_3$ for 0.005 mole of amide) had been reached. This modification obviates many trial and error experiments

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(2) Information on this and related subjects is reviewed by W. M. Whaley and T. R. Govindachari and by W. J. Gensler, in "Organic Reactions," Vol. VI, John Wiley and Sons, Inc., New York, N. Y., 1951, pp. 74–206.

(3) A. P. Dunlop and F. N. Peters, "The Furans," Reinhold Publ. Corp., New York, N. Y., 1953, pp. 640–658.