The residue remaining after removal of the methanol and N,N-dimethylhydroxylamine was extracted with ether, dried over potassium carbonate and the solution evaporated to yield a crystalline residue. This residue was recrystallized from pentane yielding colorless plates of 3-methoxymethylindole (0.72 g., 63%), m.p. 97–98° (reported[13] 99–100°).

Sodium ethoxide and gramine oxide yielded 3-ethoxymethylindole (59%), m.p. 62–63° (reported[13] 63–64°).

(c) **Alcohols.**—Gramine oxide (1.0 g.) was refluxed with 25 ml. of isobutyl alcohol for 2 hours. Removal of the solvent yielded a pale orange oil which was chromatographed on alumina. The eluting solvents varied from ether–pentane (1:3) to pure ether. The first fractions were liquid and were distilled (120° (0.0005 mm.)) to yield 3-isobutoxymethylindole, a colorless oil, $n^{25}D$ 1.5574.

Anal. Calcd. for $C_{13}H_{17}NO$: C, 76.81; H, 8.43; N, 6.89. Found: C, 76.47; H, 8.50; N, 6.84.

The 1,3,5-trinitrobenzene derivative crystallized in yellow prismatic microscopic needles, m.p. 103–104°.

Anal. Calcd. for $C_{13}H_{17}NO \cdot C_6H_3N_3O_6$: C, 54.80; H, 4.84. Found: C, 54.91 H, 4.89.

3-Isobutoxymethylindole also was obtained in 44% yield by the treatment of gramine with ethyl iodide and sodium isobutoxide. Later fractions from the alumina column were semi-solid and sublimation (80° (0.0002 mm.)) followed by crystallization from pentane yielded colorless rhombic crystals of O-skatyl-N,N-dimethylhydroxylamine, m.p. 93–94°.

On refluxing gramine oxide in ethanol, 3-ethoxymethylindole and II were obtained along with material insoluble in ether, presumably polymeric.

(d) **Sodium Hydroxide.**—Gramine oxide (1.0 g.) in 50 ml. of water was added dropwise to a rapidly stirred, refluxing mixture of 50 ml. of 10% aqueous sodium hydroxide and 50 ml. of ether. After 4 hours the ether layer was separated, dried and evaporated. The residue (0.11 g.) was crystallized from pentane to yield colorless plates of 3-hydroxymethylindole, m.p. 99–100° (reported[20] 100–101°). If the reaction was allowed to proceed overnight, more 3-hydroxymethylindole was obtainable from the ether layer.

(e) **Water.**—Gramine oxide (1.0 g.) in 20 ml. of water was heated at 100° for 20 hours. A white precipitate was produced. An ether extract of this mixture was chromatographed on alumina eluting with ether–pentane (1:4). The main fraction was crystallized from benzene–petroleum ether yielding colorless plates of 3,3′-diindolylmethane (0.10 g.), m.p. 163–164° (reported[8a] 163–164°). There was much material insoluble in ether, presumably polymeric. In a duplicate experiment, the aqueous solution at the end of the reaction was treated with dimedone yielding formaldehyde-dimedone derivative, m.p. 190–191° (58%), not depressed on admixture with an authentic specimen.

(f) **Sodium Cyanide.**—Gramine oxide (1.0 g.) in 25 ml. of water was stirred rapidly with a mixture of 25 ml. of toluene, 10 g. of sodium cyanide and 25 ml. of a saturated solution of sodium cyanide at 100° for 1.5 hours. The toluene layer was separated and dried over potassium carbonate. The liquid residue remaining after removal of the toluene *in vacuo* was dissolved in methanol and treated with 1,3,5-trinitrobenzene yielding orange needles of the 1,3,5-trinitrobenzene derivative of 3-cyanomethylindole (1.36 g., 83%), m.p. 135.5–137°.

Anal. Calcd. for $C_{10}H_8N_2 \cdot C_6H_3N_3O_6$: C, 52.03; H, 3.00. Found: C, 52.01; H, 3.12.

When gramine oxide was treated with methanolic sodium cyanide a mixture of 3-cyanomethylindole and 3-methoxymethylindole was obtained.

(g) **Nitromethane.**—Gramine oxide (1.0 g.) in 15 ml. of nitromethane was treated with a solution of sodium (0.10 g.) in 2 ml. of ethanol and refluxed with stirring for one hour. Water (0.2 ml.) was then added and the excess nitromethane removed *in vacuo*. The residue was extracted with ether, diluted with pentane and chromatographed on alumina of low activity. The major fraction from the chromatogram was crystallized from pentane, yielding colorless plates of 3-(2-nitroethyl)-indole (0.27 g., 31%), m.p. 53.5–54° (reported[21] as existing in dimorphic forms, m.p. 56.5–57° and 68–68.5°). The infrared spectrum of 3-(2-nitroethyl)-indole in chloroform has absorption bands at 1546 and 1380 cm.$^{-1}$ assignable to the NO_2 group.

(h) **Hydrochloric Acid.**—Gramine oxide (1.0 g.) in 15 ml. of water was treated with 1 ml. of 4 *N* hydrochloric acid and heated at 100° for 2 hours. The white amorphous precipitate which was obtained was filtered off, washed with water and dried in a desiccator over sodium hydroxide.

Anal. Found: C, 79.46; H, 5.47; N, 6.78. Calcd. for C_9H_7N (the empirical formula of the polymer obtained by the action of acid on 3-hydroxymethylindole[8a]): C, 83.69; H, 5.46; N, 10.85.

The aqueous filtrate was evaporated to dryness yielding a colorless sirup which was treated with picric acid giving a yellow precipitate. After several recrystallizations from ethanol, yellow needles of N,N-dimethylhydroxylamine picrate separated, m.p. 160–161°, not depressed on admixture with an authentic specimen.

Attempts to make a picrate of gramine oxide by addition of ethanolic picric acid to an ethanol solution of the oxide resulted in the formation of resinous material.

(20) J. Thesing, *Ber.*, **87**, 692 (1954).

(21) W. E. Noland and P. J. Hartmann, THIS JOURNAL, **76**, 3227 (1954).

LOS ANGELES 24, CALIF.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF WISCONSIN]

The Alkaloids of *Rauwolfia*. Structure of Raunescine and Isoraunescine

BY EUGENE E. VAN TAMELEN AND CHARLES W. TAYLOR

RECEIVED MARCH 30, 1957

The technique of gradient-elution chromatography has been applied to a mixture of minor alkaloids derived from *Rauwolfia tetraphylla* L. The generalized structure VII for two of the components, raunescine and isoraunescine, is supported by the conversion of deserpidine to raunescic acid (= isoraunescic acid), supplemented by evidence for the stereochemistry at C-3 and C-16.

Rauwolfia tetraphylla L. serves as an important commercial source of the hypotensive and tranquilizing agent reserpine, which is accompanied in this plant by a profusion of other indole alkaloids.[1,2] There became available to us substantial quantities of the alkaloid mixture[3,4] from which reserpine and certain of the other bases had been largely removed, thus affording the opportunity to scru-

(1) R. E. Woodson, Jr., H. W. Youngken, E. Schlittler and J. A. Schneider, "Rauwolfia: Botany, Pharmacognosy, Chemistry and Pharmacology," Little, Brown and Co., Boston, Mass., 1957, pp. 54–55.

(2) (a) C. Djerassi, M. Gorman, S. C. Pakrashi and R. B. Woodward, THIS JOURNAL, **78**, 1259 (1956); (b) M. W. Klohs, F. Keller, R. E. Williams and G. W. Kusserow, *Chemistry & Industry*, 187 (1956).

(3) Reserpine-depleted weakly basic alkaloids, supplied by S. B. Penick and Co., New York, N. Y.

(4) Although *R. canescens* L. and *R. heterophylla* Roem. and Schult. are botanically indistinguishable (ref. 1, p. 11) from *R. tetraphylla* L., the first term has been generally applied to Indian roots and the second, to the American alkaloid source. In this investigation a mixture from material classified as "*heterophylla*" was used.

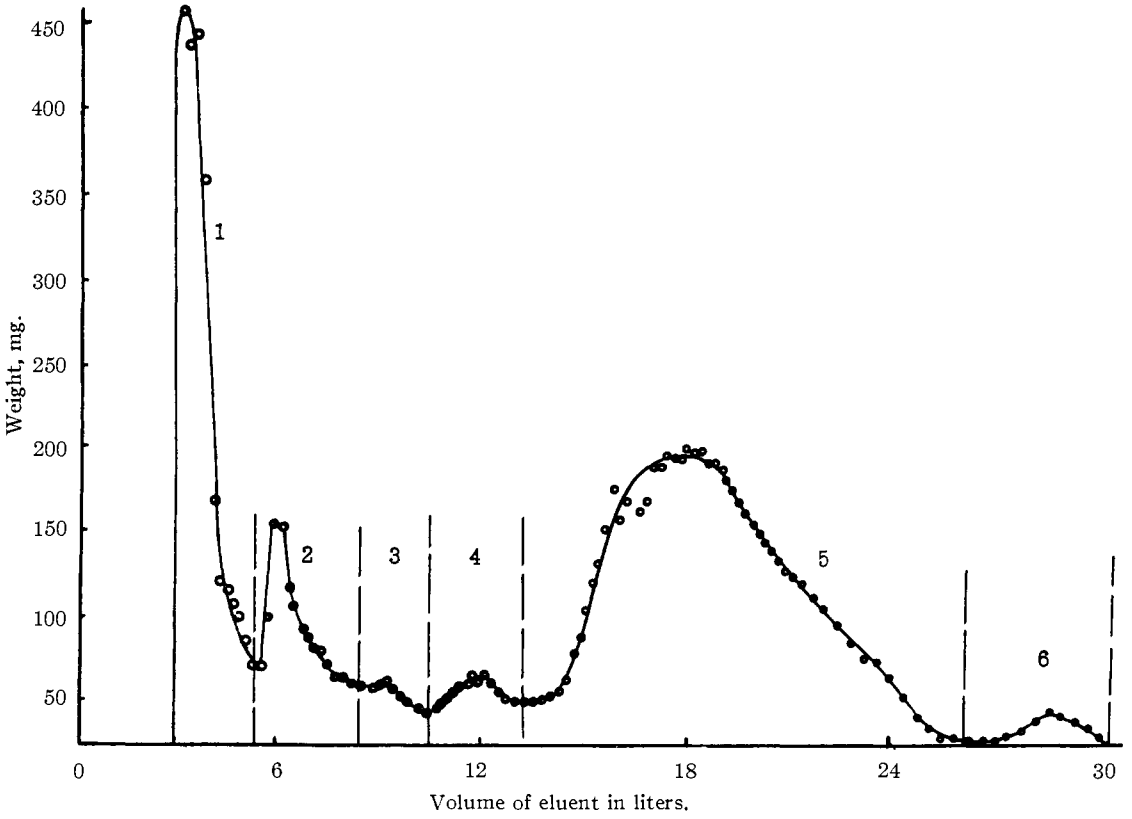


Fig. 1.

tinize the minor components of this *Rauwolfia* species. In this contribution we report results obtained in the first stages of this isolation and structure determination program.

As received from the commercial source, the minor alkaloid concentrate from *tetraphylla* extracts was a dark brown, amorphous powder, clearly a complex mixture, as evidenced by infrared spectra and paper chromatograms. Initial trials showed that normal chromatographic procedures, involving *discontinuous* changes in the ratio of the solvents used in combination for column development, was almost completely ineffective for separation purposes. By contrast, reasonably satisfactory separation was achieved by employing the gradient elution technique,[5] *i.e.*, changing, in a *continuous* fashion, the proportion of the solvents used for elution (this expedient, which can allow a degree of resolution unattainable through ordinary elution methods, has heretofore not been applied, as far as we know, to the separation of indole alkaloids). Using ethanol–benzene, varying within the range 0–40% of the former solvent, elution was carried out by means of either the two conical reservoir (for large scale operation) or the Kenyon[6] technique (for small scale runs). In order to avoid too rapid elution in the early stages as well as broadening and extensive "tailing" of the peak fractions in the later stages, we employed a nonlinear gradient in which the rate of change in the ethanol–benzene ratio is gradually increased at some appropriate rate. The results are evaluated by comparing weight of solid per unit volume of eluent to the accumulated volume of eluent, as shown graphically in Fig. 1. It soon became evident, however, that the discrete bands obtained belied the heterogeneous nature of the peak fractions, and further chromatography was often advisable, and even necessary, for the isolation of crystalline constituents. At the same time, the alkaloids identified herein, all previously described, represent only a fraction of the total material present.

Table I lists the alkaloids corresponding to particular bands in Fig. 1; the details of the isolation, purification and identification are given in the Experimental section.

Band 5, which accounted for over 40% of the total weight of recovered material, deposited, on removal of chromatographic solvent, crystalline reserpine. After this alkaloid had been removed as well as possible by crystallization techniques, the mixture recovered from the mother liquors was rechromatographed over alumina. The graph obtained featured one sharp, leading band (5'a), followed by a broader maximum (5'b) accompanied by a shoulder in the tail fractions. All attempts to secure crystalline material from bands 3 and 5'a failed. Although there was no evidence of a peak beyond the region associated with the raunescine isolation, crystalline pseudoreserpine was obtained from fractions eluted over the range 20–40% ethanol.

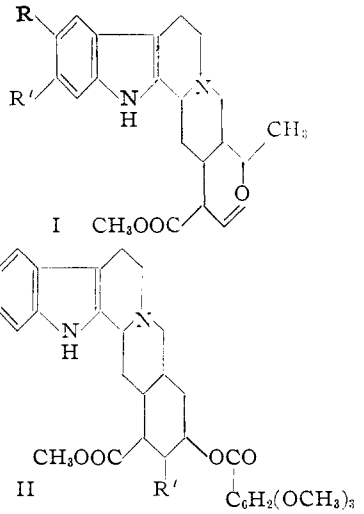
(5) R. J. P. Williams, *The Analyst*, **77**, 905 (1952).

(6) W. C. Kenyon, J. E. McCarley, E. Boucher, A. E. Robinson and A. Wielie, *Anal. Chem.*, **27**, 1888 (1953).

TABLE I

Band number	Alkaloid isolated
1	Aricine (I, R = OCH_3, R′ = H)
2	Isoreserpiline (I, R = R′ = OCH_3)[a]
4	Ajmalicine (I, R = R′ = H)
5	Reserpine (II, R = R′ = OCH_3)
5b	Deserpidine (II, R = H; R′ = OCH_3)
6 (5)	Raunescine
Region beyond 6	Pseudoreserpine

[a] Identification tentative

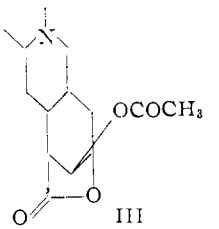


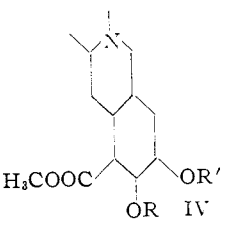
It is of interest to examine, in passing, the elution pattern of the indole alkaloids described. The first identifiable compounds eluted are the hetero ring-E class (I). These are followed by the C-17 methoxyl, C-18 trimethoxybenzoyl type (II), which just precedes raunescine and pseudoreserpine, both of which possess hydroxyl rather than methoxyl in the E-ring (*vide infra*). It seems reasonable that alkaloids as yet unisolated from any one peak fraction will resemble structurally the alkaloids already identified as components of the peak.

Raunescine and isoraunescine, possessing the molecular formulas $C_{31}H_{36}N_2O_8$, were first isolated and characterized by Hosansky and Smith.[7] Preliminary chemical observations, analytical and spectral data, as well as the nature of the observed physiological activity, led these workers to suggest that the structures of this isomeric pair were equivalent to that of deserpidine, with the difference that they lacked the methyl group on oxygen at C-17. This proposal, although unexceptional, was eminently reasonable; for example, raunescine and isoraunescine are almost indistinguishable from deserpidine in the 2–16 μ region of the infrared. The results outlined below confirm in detail the general constitution advocated by Hosansky and Smith.

One less O-methyl group in both raunescine and isoraunescine suggested the presence of hydroxyl in each member of the pair; this feature was confirmed by formation of an O-acetyl derivative in each case.[7] Basic hydrolysis liberated, as expected, one mole of 3,4,5-trimethoxybenzoic acid.[7] The presence of the carbomethoxyl group, characteristic of almost all indole alkaloids in the series, was indicated by the liberation, on complete basic hydrolysis, of one mole of methanol.

Because of the anticipated relationship between deserpidine[8] and the pair of new alkaloids, conversion of one series to the other offered the simplest approach to proof of structure. The desired juncture was effected through ether cleavage of deserpidine, carried out with constant boiling hydrobromic acid at 105°. After mild acetylation of the crude reaction product, there was obtained the acetate of a γ-lactone (infrared absorption at 5.68 μ), which can be formulated as III. Alternately, if the product from the hydrobromic acid treatment was methylated with diazomethane before





acetylation, the diacetate (IV, R = R′ = $COCH_3$) of the methyl ester was isolated. This same pair of substances also could be derived from both raunescine and isoraunescine. Complete hydrolysis of either alkaloid, carried out in aqueous methanolic potassium hydroxide, afforded the crystalline salt of raunescic acid (= isoraunescic acid), which after treatment with acetic anhydride–pyridine, furnished a crystalline product identical with the lactone acetate III described above. If the alkaloids are subjected to the action of anhydrous sodium methoxide in methanol, 3,4,5-trimethoxybenzoic acid is liberated by transesterification, and methyl raunescate (= methyl isoraunescate), an uncrystallizable glass, is formed; acetylation converted this material to a well-defined diacetate, indistinguishable from the diacetate derived from deserpidine. These results define the gross structure of methyl raunescate as IV (R = R′ = H) and allow the formulation of raunescine and isoraunescine as mono-O-3,4,5-trimethoxybenzoates of this pentacyclic system.

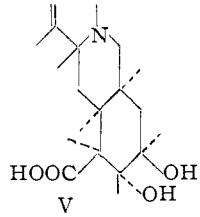
In regard to the stereochemistry of raunescine and isoraunescine it is evident that the correlation accomplished provides a secure foundation for detailed analysis, in that the relationship between all the asymmetric centers in deserpidine has been established.[8,9] The hydrobromic acid treatment employed would not be expected to disturb centers 15, 16, 17, 18 or 20 of deserpidine,[8] and consequently the stereochemistry of O-acetylraunescic acid lactone and methyl O-diacetylraunescate duplicates that of deserpidine at these centers. By contrast, members in this series which bear a C-2, C-3 axial bond, as does deserpidine, can be epimerized at C-3 by acids at elevated temperatures.[8] Now, as intimated above, the conversion of rau-

(7) N. Hosansky and E. Smith, *J. Amer. Pharm. Assoc. (Scient. Ed.)*, **44**, 639 (1955).

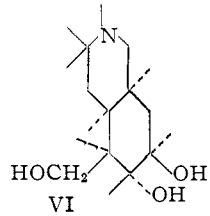
(8) H. B. MacPhillamy, C. F. Huebner, E. Schlittler, A. F. St. Andre and P. R. Ulshafer, THIS JOURNAL, **77**, 4335 (1955).

(9) (a) C. F. Huebner and E. Wenkert, *ibid.*, **77**, 4180 (1955); (b) P. A. Diassi, F. L. Weisenborn, C. M. Dylion and O. Wintersteiner, *ibid.*, **77**, 4687 (1955); (c) F. E. van Tamelen and P. D. Hance, **77**, 4692 (1955); (d) C. F. Huebner and D. F. Dickel, *Experientia*, **12**, 250 (1956).

nescic acid to the lactone III is carried out under mild conditions, *viz.*, acetic anhydride–pyridine at 0°. Correspondingly facile lactonizations are characteristic of the C-3 epi(axial) configuration in the reserpine–deserpidine series, whereas members of the C-3 iso(equatorial) group give equivalent products under only very much more stringent conditions.[10,11] The tentative conclusion that the raunescic acid derivatives possess the C-3 epi-configuration was reinforced by the stability of raunescine and isoraunescine toward mercuric acetate, a recognized diagnostic reagent for assigning C-3 configurations in the indole alkaloid series.[12,13] Formation of raunescic acid by ether cleavage of deserpidine does not involve inversion, therefore, at the C-3 position, and it is likely that the conditions employed were borderline ones, insofar as epimerization or non-epimerization is concerned.[14] With this last detail disposed of, the complete derivative is defined and is depicted by formula V.



V



VI

Raunescic acid and methyl raunescate are generated from raunescine or isoraunescine by strong base at temperatures at or below 100°, conditions which can affect but one asymmetric center, namely, C-16. Therefore the individual stereochemical assignments made in the foregoing are directly applicable to the alkaloids themselves, with the exception of the assignment at this one asymmetric center. In order to resolve the uncertainty, raunescine, isoraunescine and the lactone III were reduced by means of lithium aluminum hydride, a reagent which does not epimerize carboxylic esters. The same triol (VI) was obtained from each of the three starting carboxylic acid derivatives. The C-16 configurations of the alkaloids may then be safely regarded as identical and equivalent to that of methyl raunescate. It further follows that raunescine and isoraunescine are structurally and configurationally identical, save for the point of attachment of the trimethoxybenzoyl group, which may be at C-17 or C-18 (see formula VII).

Some time after the work described above had been completed, Huebner and Schlittler, on the basis of a largely different body of results,[15] proposed for raunescine and isoraunescine the stereoformula VII and thereby also confirmed in the main

(10) C. F. Huebner, M. E. Kuehne, B. Korzun and E. Schlittler, *Experientia* **12**, 249 (1956).

(11) R. B. Woodward, F. E. Bader, H. Bickel, A. J. Frey and R. W. Kierstead, THIS JOURNAL, **78**, 2023 (1956).

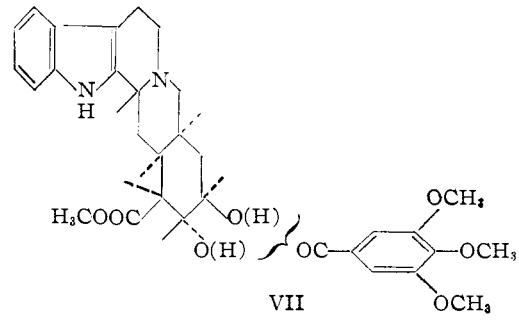
(12) F. L. Weisenborn and P. A. Diassi, *ibid.*, **78**, 2022 (1956).

(13) E. Wenkert and D. K. Roychaudhuri, *ibid.*, **78**, 6417 (1956), have reported infrared spectral evidence which also indicates the C-3 epi-configuration for raunescine and isoraunescine.

(14) The infrared determination (see ref. 13) on a second crystalline product resulting from the hydrobromic acid cleavage step disclosed that C-3 epimerization had occurred at least in part.

(15) C. F. Huebner and E. Schlittler, THIS JOURNAL, **79**, 250 (1957).

the structure suggested by Hosansky and Smith.[7] In addition, the former workers reported comparative esterification rates which led them to assign the position of the trimethoxybenzoyl group in each of these alkaloids. The points of attachment were not regarded by these authors as "established



VII

with the same certainty as the structure of the alkanol amine, methyl raunescate"; on the other hand, we have not acquired evidence on this matter which we consider more convincing than that broached by the Ciba group.

Acknowledgment.—The authors are grateful to W. G. Bywater and E. Smith of S. B. Penick and Co. for support and coöperation, and to Dr. John Dyer for valuable advice on chromatographic procedures.

Experimental[16]

Physical Properties of the Alkaloid Mixture. Solubility.—The mixture was extremely soluble in chloroform and dioxane and reasonably soluble in methanol and ethanol. It exhibited limited solubility in benzene (half of a 1-g. sample of the alkaloid mixture was soluble in 85 ml. of dry benzene) and was virtually insoluble in ether and petroleum ether.

Spectral Properties.—The ultraviolet spectrum exhibits a maximum at 268 mμ (E_1^1 215) and a point of inflection at 289 mμ. The infrared spectrum showed carbonyl absorption (5.80–5.88 μ) and some aromatic absorption between 6–7 μ, but beyond 7 μ the absorption was completely lacking in detail.

Other Physical Properties.—The mixture was a brown, powdery, amorphous solid which in solution exhibited some fluorescence in daylight and enhanced fluorescence under ultraviolet light (blacklight).

All attempts to decolorize solutions of the mixture failed, and chromatography of the material invariably resulted in a more or less uniform distribution of the color throughout the separation.

Techniques Employed with Alumina Chromatography.—Initial chromatographic experiments using acid-washed alumina indicated that if the technique was to be used successfully to separate the alkaloid mixture and yield interpretable results, the solvent polarity would have to be changed in a uniform fashion. It was particularly true in this case because of the small structural differences in the components of the mixture and because of the relatively high proportion of residue that accompanied the elution. A number of methods can be devised for accomplishing gradient elution, two of which were used in this work.

A gradient elution technique particularly useful in relatively large scale chromatograms employed two conical reservoirs, one of which was inverted. The apparatus, as adapted to this chromatographic problem, is shown in Fig. 2. Both conical reservoirs are open to the air, and since there is a liquid bridge between them, the levels in the two reservoirs must always be the same (neglecting small density differ-

(16) All melting points were corrected and, unless otherwise stated, were taken in sealed, evacuated capillaries. The infrared spectra were taken on a Baird infrared recording spectrometer (model B). The ultraviolet spectra were taken on a Cary recording spectrophotometer (model 11 MS) in 95% ethanol using a 1-cm. cell.

ences between the solvents). The less polar solvent is always contained in the inverted reservoir and, when the solvent system is refilled, the inverted reservoir is filled with solvent of the same polarity as that previously contained in the upright conical reservoir. In this way there is no discontinuity in the solvent gradient. Irregularities arising from diffusion or mixing due to density differences in the solvents are relatively unimportant unless solvents of greatly differing densities (*e.g.*, chloroform and alcohol) are used.

It was found that the "tap-off" at the top of the siphon from the upright erlenmeyer flask was necessary. If such an outlet was not provided, dissolved gas in the solvent soon formed bubbles which partially broke the siphon and allowed unequal drainage from the system. This tap also provided an easy method for starting the siphon.

A second gradient elution apparatus[6] was used for smaller columns.

Although fractions were manually cut in some of the preliminary chromatographic work, a Reco Fraction Collector (Timer/Controller Model F1200 A) was employed for the separations described here. Equal volume fractions were taken in all cases except the one column in which chloroform was the developing solvent.

The tubes from the fraction cutter were combined (the number of tubes combined depended partly upon previous experience and partly upon the weight of material in the preceding fractions) and the solvent removed *in vacuo* below 50°. When the volume was reduced to a few ml., the material was transferred to a tared 10-ml. erlenmeyer and the remaining solvent evaporated with nitrogen. Finally, the fractions were thoroughly dried under high vacuum and their weights recorded and plotted against volume of eluent. If the fractions were kept for any length of time, they were stored under nitrogen.

Initially an attempt was made to follow the development of the column with an ultraviolet lamp (blacklight), but it soon became apparent that the most highly fluorescent materials were generally impurities and that there was no relationship between the fluorescent bands and the bands obtained on a weight basis; therefore, this technique was abandoned.

Chromatographic Separation of the Alkaloid Mixture on Acid-washed Alumina.—Alkaloid mixture (35 g.) was stirred for 48 hr. with 3 l. of dry benzene. The insoluble material (17.6 g.) was filtered off, leaving 17.4 g. of soluble alkaloid mixture. This solution was applied to 2965 g. of acid-washed alumina in a column (5 × 160 cm.). The column was developed, the elution proceeding at a rate of 4 ml. per minute. The weights of material in each band and the percentages of the total mixture are recorded in Table II.

TABLE II

SUMMARY OF RESULTS OF ACID-WASHED ALUMINA CHROMATOGRAM

Band	Fractions	Weight in mg.	% of mixture applied to column
Forerun	1	9	
1	2–12	2,445	14.0
	13–14	143	
2	15–25	1,113	6.4
	26–28	175	
3	29–38	551	3.1
	39	43	
4	40–53	808	4.6
	54–57	189	
5	58–104	7,378	42.5
	105–106	89	
6	107–117	757	4.3
	118–150	1,231	7.0
Total		14,930	85.7

Band 1.—The major portion (2.0 g.) of band 1 was rechromatographed on 50 g. of silicic acid in a column (3.1 × 15 cm.). The main band (1.9 g.) was eluted with 3% methanol in chloroform. A small highly colored initial band (20 mg.) and three small colored bands (100 mg.) accounted for the remaining material eluted. Part of the main band (870 mg.) was dissolved in 5 ml. of methanol and treated, while cooling in an ice-bath, with 10 drops of methanolic nitric acid (1 part concentrated nitric acid and 1 part methanol). Within a few minutes a crystalline substance separated from solution. This material was filtered, washed four times with 2-ml. portions of methanol and then recrystallized from absolute methanol to yield 180 mg. of shiny platelets of the nitrate, m.p. 263–265° dec.

The nitrate salt (130 mg.) was suspended in 1 ml. of water and treated with 3 drops of concentrated ammonium hydroxide. The resulting solution was extracted three times with 1-ml. portions of chloroform, and the combined chloroform extracts were washed with water, dried and the solvent evaporated with nitrogen leaving 100 mg. of white amorphous solid. After two recrystallizations of this material from methanol–water, 85 mg. of highly crystalline aricine was obtained, m.p. 186–187°, mixed m.p. with an authentic aricine, 185–187°, $[\alpha]^{21}D$ −90.6° (chloroform). The infrared and ultraviolet spectra of this material were identical with those of authentic aricine.[1]

When the remainder of the band, in methanol–water, was seeded, an additional 156 mg. of aricine was obtained.

Band 2.—The combined fractions from band 2 (1.1 g.) were rechromatographed on 28 g. of silicic acid using a chloroform–methanol gradient 0.1–10.0% of methanol in chloroform. The bulk of the material (0.7 g.) came off in a sharp band. This material was dissolved in absolute methanol and treated very cautiously, while cooling, with two drops of methanolic nitric acid. The solution turned very dark but a small amount of crystalline nitrate salt separated. This material was centrifuged and the dark solution was drawn off. After washing three times with cold methanol and recrystallizing once with absolute methanol, a small amount (6 mg.) of white crystalline nitrate salt was obtained which exhibited an ultraviolet spectrum identical with that of isoreserpiline. Regeneration of the free base was accomplished by treating an aqueous suspension of the salt with ammonium hydroxide and then extracting the aqueous phase with chloroform. After washing the chloroform extracts with water, drying over anhydrous sodium sulfate and blowing off the solvent with nitrogen, 5 mg. of a light amber glass remained. The infrared spectrum of this material was identical with that of isoreserpiline.

Band 3.—No crystalline material could be obtained from band 3, either directly or through attempted nitrate salt formation.

Band 4.—Upon standing, the fractions from band 4 slowly deposited a crystalline material from an ethanol solution. After recrystallization from ethanol and then ethyl acetate, a white, crystalline material was obtained, the ultraviolet and infrared spectra of which were identical in every respect to those of ajmalicine, m.p. 261–263°, mixed m.p. with authentic ajmalicine, 261–263°, $[\alpha]D$ −46.6° (pyridine).

Band 5.—The material from band 5 yielded 3.3 g. (19% of mixture applied to the column) of crystalline reserpine from an ethanol solution, m.p. 264.5–266° (open capillary), mixed m.p. with authentic reserpine, 264.5–266° (open capillary).

Band 6.—Using material from a run involving 105 g. of alkaloid (benzene-soluble), the fractions were combined (4.00 g.) and rechromatographed on 800 g. of acid-washed alumina starting with 20% chloroform in benzene and gradually increasing the polarity with ethanol. An initial band (0.6 g.) followed by a large band (2.4 g.) was obtained. The fractions comprising the latter portion of the large band yielded crystalline material from an aqueous methanolic solution. This light brown solid (592 mg.) was filtered off and recrystallized from aqueous methanol yielding 454 mg. of crystalline solid exhibiting ultraviolet and infrared spectra identical with those of raunescine, m.p. 160–170°.

A nitrate salt of this material and a nitrate salt from authentic raunescine were prepared, both of which melted at 208–210° dec.

The fractions following the raunescine band (trailing fractions eluted with 20–40% ethanol in benzene and showing no evidence of a band on a weight basis) yielded crystalline material from ethanol solution. This light brown solid was filtered (1.37 g.) and recrystallized twice from aqueous acetone to give 620 mg. of nicely crystalline material, m.p. 256–257°, mixed m.p. with authentic pseudoreserpine, 254–255°, $[\alpha]D$ −61° (chloroform.) The ultraviolet and infrared spectra of this material were identical with those of pseudoreserpine.

Rechromatography of the Reserpine Mother Liquors.—The combined mother liquors after removal of 0.408 g. of ajmalicine and 20.66 g. of reserpine were freed of solvent (23 g. of residue) and then dissolved in 1 l. of dry benzene. This solution was applied to 2940 g. of acid-washed alumina in a column (5 × 152 cm.). The column was developed with 50 l. of solvent embodying a polarity change of 0 to 35% ethanol in benzene. The first band resisted all attempts at crystallization; however, the minimal region immediately following this band did yield a small quantity of crystalline material exhibiting an ultraviolet spectrum possessing strong broad absorption between 300 and 360 mμ.

The fractions comprising the broad second band were dissolved in ethyl acetate and combined. After seeding with deserpidine, crystalline material separated from solution and was filtered off (1.72 g.). After recrystallization from ethyl acetate 1.27 g. of white crystalline solid was obtained, the ultraviolet and infrared spectra of which were identical with those of authentic deserpidine (similar to reserpine but lacking the band at 6.13 μ attributed to the 11-methoxyl) m.p. 224–226°.

A nitrate salt was prepared from this material and recrystallized from absolute methanol, m.p. 252–254°. This material was considerably different from the nitrate salt of reserpine which melted at 264–267°.

The shoulder region following the deserpidine peak yielded reserpine from ethanol solution.

Chromatography of the Benzene-insoluble Alkaloidal Material.—The residue remaining after extracting 8.4 g. of alkaloid mixture with 720 ml. of dry benzene (4.2 g.) was dissolved in 50 ml. of chloroform (washed with concentrated sulfuric acid, dried over calcium chloride and distilled) and applied to 850 g. of acid-washed alumina in a column (3.2 × 100 cm.). The column was developed with chloroform containing increasing amounts of ethanol (0 to 20%). Only 34% of the material applied to the column could be eluted, this being accounted for in one major band, preceded by a shoulder and followed by a plateau that very gradually decreased to zero. A small amount of crystalline reserpine was obtained from the major band and the plateau region yielded a small amount of crystalline pseudoreserpine, but no further crystalline substances could be isolated.

Methyl Isoraunescate.—Isoraunescine, 300 mg. (0.53 mole), was suspended in 30 ml. of absolute methanol containing 30 mg. of dissolved sodium. The mixture was maintained at reflux for 1 hr. (complete solution of isoraunescine had occurred after 15 minutes) under nitrogen. The solution was then concentrated to approximately 3 ml. by evaporation under a jet of nitrogen, diluted to 20 ml. with water and acidified to *p*H 2 with hydrochloric acid. This mixture was extracted three times with 5-ml. portions of ether to remove methyl 3,4,5-trimethoxybenzoate and then made basic to *p*H 10 with ammonium hydroxide. This basic mixture was extracted with several portions of chloroform, and the combined chloroform extracts were washed twice with water and dried over magnesium sulfate. A pale yellow glass, 147 mg. (78%), was obtained upon removal of solvent *in vacuo*.

Potassium Salt of Isoraunesic Acid.—Isoraunescine, 203 mg. (0.35 mmole), was suspended in 5 ml. of methanol and treated with 400 mg. of potassium hydroxide dissolved in 1 ml. of water. The system was flushed out with nitrogen and the reaction mixture then maintained under reflux for 1.5 hr. After this period of time the solution was concentrated to 3 ml. under a jet of nitrogen, and the white crystalline solid that separated from the solution was filtered, washed with a small amount of cold methanol and dried, affording 115 mg. (81%) of potassium isoraunescate.

Isoraunesic Acid Lactone.—Crude potassium isoraunescate, 57 mg. (0.14 mmole) (dried under high vacuum), was dissolved in 20 ml. of pyridine and treated with 0.6 ml. of freshly distilled acetic anhydride. This reaction mixture (protected from moisture) was allowed to stand at 5° for four days, after which time 10 ml. of saturated sodium bicarbonate solution was added to neutralize acetic acid, and the solvent was then removed *in vacuo*. The residue was taken up in 10 ml. of water and lyophilized to remove traces of pyridine, after which the residue was suspended in water and extracted with chloroform. The chloroform extracts were combined, washed twice with water and once with saturated sodium chloride solution and finally dried over anhydrous sodium sulfate. Removal of chloroform left

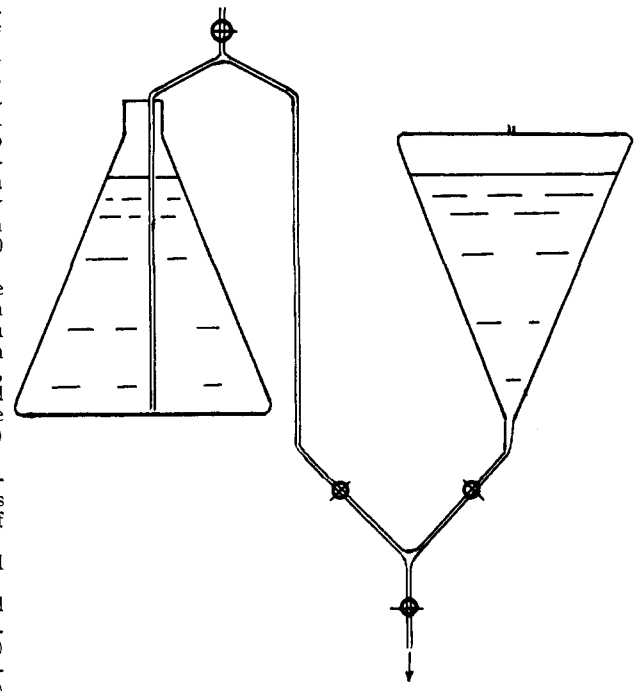


Fig. 2.

47 mg. of yellow solid which afforded, after recrystallization from isopropyl alcohol–methanol, 41 mg. (75%) of white, crystalline lactone, m.p. 272–273° dec., $[\alpha]_D$ +72.8° (pyridine).

Anal. Calcd. for $C_{22}H_{24}N_2O_4$: C, 69.45; H, 6.37. Found: C, 69.40; H, 6.27.

The infrared spectrum of this material exhibited characteristic γ-lactone absorption at 5.68 μ.

Methyl Raunescate, Potassium Raunescate and Raunesic Acid Lactone.—The transesterification, hydrolysis and lactonization experiments described for isoraunescine were carried out in exactly the same manner on raunescine. The yields in all cases were comparable.

Raunesic acid lactone was obtained from potassium raunescate in 64% yield, m.p. 271–272° dec., $[\alpha]_D$ +75.5° (pyridine). The ultraviolet and infrared spectra of raunesic acid lactone and isoraunesic acid lactone were identical.

Methyl Isoraunescate Diacetate.—To a solution of 168 mg. (0.45 mmole) of methyl isoraunescate in 10 ml. of pyridine, 1.5 ml. (15 mmoles) of acetic anhydride slowly was added with cooling. After standing at 5° for four days, the reaction mixture was treated with saturated sodium bicarbonate solution to destroy excess acetic anhydride and to neutralize acetic acid. The solvent was removed *in vacuo* and the residue lyophilized from water to remove traces of pyridine, after which the residue was suspended in water and extracted several times with chloroform. The chloroform extracts were combined, washed twice with water and once with saturated sodium chloride solution and then dried over anhydrous magnesium sulfate. Removal of chloroform left 180 mg. of light amber froth. Crystallization of this material from isopropyl alcohol yielded white platelets of the diacetate, m.p. 276–277° dec., $[\alpha]_D$ −114.5° (chloroform).

Anal. Calcd. for $C_{25}H_{30}N_2O_6$: C, 66.06; H, 6.65; CH_3CO, 18.9. Found: C, 66.50; H, 6.84; CH_3CO, 18.3.

Ether Cleavage of Deserpidine with Hydrobromic Acid.—Deserpidine, 805 mg. (1.4 mmoles), was suspended in 10 ml. of freshly distilled hydrobromic acid (48% by titration). The system was completely flushed out with nitrogen and the reaction mixture then heated at 105°, with occasional swirling, for 90 minutes. The mixture became homogeneous within 15 minutes after heating was begun. When the reaction mixture had cooled, the solvent was removed *in vacuo* and the residue taken up in a minimum amount of water and lyophilized to remove remaining traces of hydrobromic acid. This material was again dissolved in water

and extracted with four portions of ether to remove non-basic material. Finally, the solvent from the aqueous solution was removed under vacuum leaving an amber froth.

Methylation and Acetylation of the Deserpidine Cleavage Product.—The amorphous solid from the ether cleavage of 805 mg. of deserpidine (above) was dissolved in absolute methanol and treated with a large excess of ethereal diazomethane. Additional methanol was added to prevent ether-insoluble material from separating from the solution. This mixture was allowed to stand for 1 hr., after which time the excess diazomethane was destroyed with acetic acid. The solvent was evaporated under nitrogen and the residue vacuum dried.

Acetylation was effected by dissolving this amber amorphous solid in 10 ml. of pyridine and adding, with cooling, 2 ml. of redistilled acetic anhydride. The reaction mixture was allowed to stand at room temperature for 40 hr., after which time aqueous sodium bicarbonate was added to destroy excess acetic anhydride and neutralize acetic acid. The solvent was then removed *in vacuo* and the residue was taken up in water and extracted repeatedly with small portions of chloroform. The chloroform extracts were washed with water and dried over anhydrous sodium sulfate. The amber glass remaining after removal of solvent, 690 mg., was dissolved in 6 ml. of 20% chloroform in benzene and applied to 70 g. of Merck acid-washed alumina in a column (1 × 67 cm.). The chromatogram was developed with 20% chloroform in benzene containing increasing amounts of ethanol. Two bands were obtained, the first being eluted with 5% ethanol in the chloroform–benzene solvent and the second with 25% ethanol in this solvent. The first band yielded, from ethanol, 103 mg. of fine needles, the infrared spectrum of which exhibited two distinct peaks on the long wave length side of the C–H band at 3.46 μ, indicating that epimerization had occurred during demethylation.[13] The fractions from the second band were combined and recrystallized three times from isopropyl alcohol giving 110 mg. of crystalline platelets, m.p. 276–277° dec., mixed m.p. with methyl raunescate diacetate 276–277° dec., $[\alpha]_D$ −115.6° (chloroform). The infrared (in chloroform and potassium bromide pellet) and ultraviolet spectra of this material were identical with those of methyl isoraunescate diacetate.

Formation of a γ-Lactone from the Ether Cleavage Product of Deserpidine.—The crude hydrobromide from the hydrobromic acid cleavage of 1.057 g. (1.8 mmoles) of deserpidine was dissolved in water and made basic to *p*H 9 with 1 *N* potassium hydroxide followed by immediate removal of the solvent by lyophilolysis. The dry residue was dissolved in 200 ml. of pyridine and treated, while cooling, with 10 ml. of redistilled acetic anhydride, after which the reaction mixture was allowed to stand for five days at 5°. The acetic acid and excess acetic anhydride were then destroyed with saturated sodium bicarbonate and the solvent removed *in vacuo*. The residue was suspended in water and extracted repeatedly with chloroform. The chloroform extracts were combined, washed with water followed by saturated sodium chloride solution and finally dried over anhydrous sodium sulfate. Removal of chloroform left 237 mg. of crude crystalline solid. Four recrystallizations of this material from isopropyl alcohol afforded 103 mg. of crystalline lactone, m.p. 271–272° dec. The infrared spectra of this material, in chloroform and in potassium bromide pellet, were identical with the corresponding spectra of raunesic acid lactone and isoraunesic acid lactone.

Raunescinetriol.—Isoraunescine, 191 mg. (0.34 mmole), was dissolved in 35 ml. of tetrahydrofuran and dropped slowly into a stirred suspension of 760 mg. of lithium aluminum hydride in 25 ml. of ether. When the addition was complete the magnetically stirred reaction mixture was maintained under reflux for 4 hr. After cooling, 5 ml. of ethyl acetate was added carefully to decompose the excess lithium aluminum hydride, and then 10 ml. of saturated aqueous sodium sulfate solution was introduced to decompose the organic salts. The suspension was centrifuged, the supernatant solution removed and the residual salts extracted several times with liberal quantities of the same solvent. The tetrahydrofuran–ether extracts were combined, dried over anhydrous sodium sulfate and the solvent removed leaving 192 mg. of yellow amorphous solid. This material was taken up in 1.5 ml. of absolute methanol, centrifuged to remove a small amount of suspended material and then treated while cooling in an ice-bath with methanolic nitric acid (equal volumes of methanol and concentrated nitric acid). A solid separated from solution and was filtered off, washed with cold methanol and dried, giving 81 mg. of crystalline nitrate salt. This material was recrystallized from absolute methanol to give a sample melting at 278.5–279.5° dec., $[\alpha]_D$ −19.1° (pyridine).

Anal. Calcd. for $C_{20}H_{26}N_2O_3 \cdot HNO_3$: C, 59.27; H, 6.71. Found: C, 59.12; H, 6.61.

A portion of this nitrate salt was suspended in water, treated with ammonium hydroxide and, after trituration to assure complete regeneration of the free base, the solid was filtered and recrystallized from water affording feathery needles of isoraunescinetriol.

Raunescine was reduced with lithium aluminum hydride in exactly the same way as isoraunescine (above). The nitrate salt obtained was recrystallized from absolute methanol, m.p. 277–279° dec., mixed m.p. with the nitrate of isoraunescinetriol 277–279°. The infrared spectrum of the nitrate salt as well as that of the regenerated triol-base (both in potassium bromide pellet) were identical with the corresponding spectra of isoraunescinetriol nitrate and free base.

Reduction of Isoraunesic Acid Lactone.—The lithium aluminum hydride reduction of isoraunesic acid lactone and the subsequent work-up was exactly the same as described above for isoraunescine. The infrared spectrum (in potassium bromide pellet) of the resulting nitrate salt (recrystallized from ethanol) was identical with the corresponding spectra of isoraunescinetriol and raunescinetriol, $[\alpha]_D$ −18.8° (pyridine).

Mercuric Acetate Oxidations.—Yohimbine, reserpine, deserpidine, isoraunescine and raunescine were subjected to mercuric acetate oxidation. Each alkaloid (100 mg.) was dissolved in 15 ml. of glacial acetic acid containing 0.15 g. of mercuric acetate. These solutions were heated under nitrogen for 2 hr. at 60°. After this period of time the yohimbine reaction mixture was the only one from which mercurous acetate had deposited. The ultraviolet spectrum of an aliquot from the yohimbine reaction mixture revealed absorption in the region of 355 mμ indicative of dehydrogenation at C-3, whereas the ultraviolet spectra of the other four reaction mixtures showed no absorption in this region.

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