agent (containing suspended potassium bromide) was added with stirring 10.0 g. (0.0485 mole) of α-amino ether I in 50 ml. of ether. After stirring for 4 hours the reaction mixture was poured onto iced hydrochloric acid, and the resulting precipitate collected on a funnel (sucked dry). This crude hydrochloride was warmed on a steam-bath with an excess of sodium carbonate solution until carbon dioxide ceased to be evolved. The liberated amine was collected on a funnel, washed with water, dried, and recrystallized from petroleum ether (cooled in Dry Ice) to give 11.0 g. (76%) of tertiary amine II, m.p. 125–126°, reported m.p. 126.5–127.5°.[4] More (0.8 g.) of II, m.p. 125–126°, was isolated from the filtrate; total yield 82%. The melting point was not depressed on admixture with an authentic sample of II.[4]

When an experiment was carried out with potassium diphenylmethide and amino ether I under similar conditions, the red color was not discharged and no precipitate of the amine hydrochloride was obtained on pouring the reaction mixture into iced hydrochloric acid. A similar observation has been reported.[4]

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Chromatography of Chymotrypsin-α

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This investigation has been carried out to determine the chromatographic behavior of chymotrypsin-α on ion-exchange columns under conditions similar to those which have proved suitable for the chromatography of chymotrypsinogen.[1] The curve shown in Fig. 1 was obtained when a sample of crystalline chymotrypsin-α (Worthington Biochemical Corp., Freehold, New Jersey), prepared by the method of Kunitz,[2] was chromatographed at 25° on a 0.9 × 30 cm. column of the sodium form of Amberlite IRC-50 (XE-64) using as eluent a 0.2 *M* phosphate buffer at *p*H 6.02. The enzyme emerges at about the same effluent volume observed previously for the zymogen under the same conditions.[1] The recovery of chymotrypsin activity in the single large peak at 25 effluent ml. was quantitative within the limits of precision of the spectrophotometric assay method of Kunitz[3] in which casein is used as substrate. The recovery of ninhydrin color varied from 95 to 112%[4] when the more rapidly eluted inactive components were included. These fast moving peaks probably represent some of the split products formed during tryptic activation of chymotrypsinogen, for Rovery, Fabre and Desnuelle,[5] using the DNP technique, have demonstrated the presence in chymotrypsin-α of adsorbed peptides not removable by crystallization, dialysis, or trichloroacetic acid precipitation.

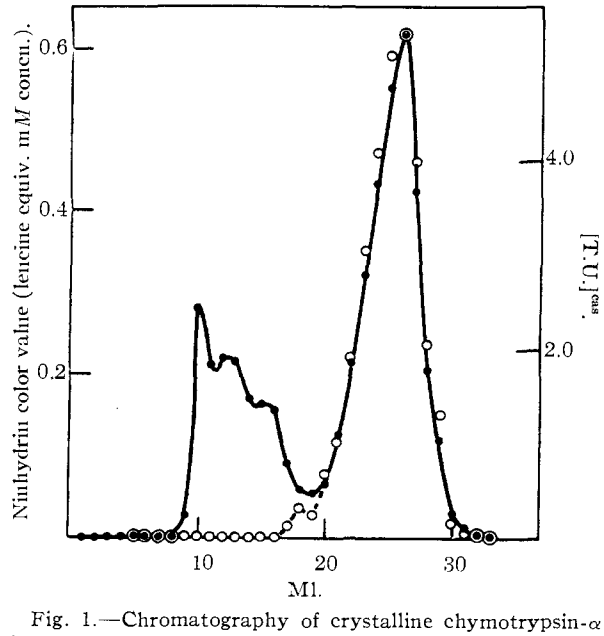

Fig. 1.—Chromatography of crystalline chymotrypsin-α (6.5 mg.) on a 0.9 × 30 cm. column of IRC-50 (XE-64). Elution was performed with a 0.2 *M* phosphate buffer at *p*H 6.02 at a rate of 2 ml. per hour. The effluent was collected in 1-ml. fractions, and aliquots were pipetted for ninhydrin analyses and for determinations of enzymatic activity (T.U. = trypsin units)[2] using Hammarsten's casein as substrate; ●—●, ninhydrin color; O—O, enzymatic activity.

Under the conditions employed for the chromatogram shown in Fig. 1, the enzyme and the zymogen would not be well separated. By the use of a slightly more acidic (*p*H 5.67) citrate buffer as elu-

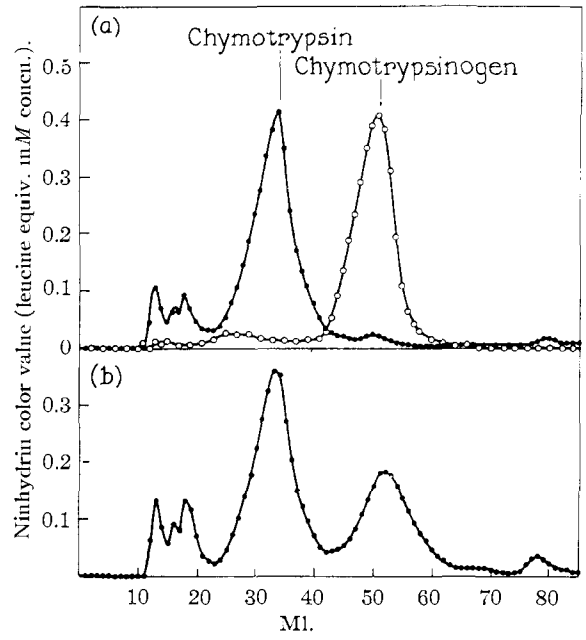

Fig. 2.—Chromatography of chymotrypsin and chymotrypsinogen on a 0.9 × 30 cm. column of IRC-50 (XE-64). Elution was performed with a citrate buffer at *p*H 5.67, 0.1 *M* in respect to citric acid and 0.255 *N* in respect to sodium, at a rate of 2 ml. per hour. The effluent was collected in 1-ml. fractions; ●—●, chymotrypsin; O—O, chymotrypsinogen. Figure 2a, chymotrypsin-α (9.0 mg.) and chymotrypsinogen (10.5 mg.) chromatographed in separate experiments; Figure 2b, chromatography of a mixture of chymotrypsin α (8.0 mg.) and chymotrypsinogen (5.7 mg.).

(1) C. H. W. Hirs, *J. Biol. Chem.*, **205**, 93 (1953).

(2) M. Kunitz, *J. Gen. Physiol.*, **32**, 265 (1948).

(3) J. H. Northrop, M. Kunitz and R. M. Herriott, "Crystalline Enzymes," Columbia University Press, New York, N. Y., 2nd Edition, 1948.

(4) Some autodigestion of the enzyme may occur during chromatography or while the effluent fractions are on the fraction collector.

(5) M. Rovery, C. Fabre and P. Desnuelle, *Biochim. Biophys. Acta*, **12**, 547 (1953).

ent, however, good separation into two well-defined peaks can be achieved, as may be seen in Figs. 2a and 2b. Phosphate buffers were found to be less satisfactory than citrate buffers. In these experiments, it is particularly important that the column be carefully equilibrated before use. Buffer should be passed through the column until the *p*H of the effluent and the influent are the same to within ± 0.02 *p*H unit before the sample (1 to 2 ml. adjusted to *p*H 5.67) is added. The results with the ion-exchange columns are very similar to those recently reported by Porter,[6] who separated the same two proteins by the use of a buffered Cellosolve–Carbitol–water system with a column of silane-treated Hyflo.

The chymotrypsin peaks are always broader than those obtained from chymotrypsinogen, and are noticeably asymmetric. Since Bettelheim and Neurath[7] have shown that chymotrypsin-α may consist of more than one active protein, the asymmetry in Figs. 2a and 2b may signify that closely related components are being partially separated.

(6) R. R. Porter, in S. P. Colowick and N. O. Kaplan, "Methods in Enzymology," Vol. I, Academic Press, Inc., New York, N. Y., 1955.

(7) F. R. Bettelheim and H. Neurath, *J. Biol. Chem.*, **212**, 241 (1955).

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Alkaloids of *Rauwolfia sellowii*

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We wish to report a brief study of the alkaloids present in the root-bark of *Rauwolfia sellowii*.[1] This plant, which is native to Brazil, has been reported to contain alkaloids.[2] The pharmacological action of the mixed alkaloids has been studied.[3] No attempt to isolate or identify the various alkaloids present appears to have been made.

A preliminary examination of the crude alkaloid extract by paper chromatography[4] suggested that ajmaline and aricin were the major components. Traces of reserpine and of other alkaloids were also indicated. The crude alkaloids were first separated into two fractions, the first consisting of those alkaloids with benzene-soluble acetates, the second containing all other alkaloids.

The first fraction yielded 1% of crystalline aricin on direct crystallization. Chromatography of the mother liquors over alumina yielded the additional pure alkaloids ajmalicine, 0.001%, reserpine, 0.002%, and tetrahydroalstonine, 0.002%. The second fraction yielded 1.35% of ajmaline on direct crystallization.

Paper chromatographic examination of various fractions and mother liquors from these isolations suggested the presence of at least four additional alkaloids, which were not identified.

(1) The plant material used in this study was kindly provided by Mr. H. R. Chaves of Instituto Vital, Niteroi, Brazil.

(2) T. A. N. de Toledo and R. Wasicky, *Scientia Farm.*, **22**, 217 (1954); *C. A.*, **49**, 5780 (1955).

(3) R. A. Sebo, J. S. Campos and J. G. Kulmann, *Rev. Quim. Farm.*, **19**, 229 (1954).

(4) F. A. Hochstein, Kotaro Murai and W. Boegemann, THIS JOURNAL, **77**, 3551 (1955).

Though alstonine has been reported as a component of several Rauwolfia species, including *R. vomitoria* and *R. obscura*,[5] and probably occurs also in *R. sellowii*, the presence of tetrahydroalstonine in a Rauwolfia species has not been previously reported.

Experimental

Extraction of Alkaloids.—Four hundred and twenty grams of *R. sellowii* root-bark, ground to pass 20 mesh, was heated under reflux with 1 liter of ethanol for 2 hr. The ethanol was separated by filtration, and the extraction was repeated twice with 1-liter portions of methanol at reflux temperature for 5 hr. The combined extracts were concentrated to dryness, *in vacuo*, to yield 61 g. of solid extract.

A crude alkaloid fraction obtained by chloroform extraction of a small portion of the basic extract was examined by paper chromatography. Ajmaline and aricin appeared to be present in large amounts, and the subsequent workup was designed to separate these two alkaloids.

The crude extract was dissolved in 200 ml. of methanol and diluted to 800 ml. with 10% aqueous acetic acid. The turbid suspension was washed once with 500 ml. of hexane, which removed 2.0 g. of non-alkaloidal oils. Extraction with four 500-ml. portions of benzene removed an alkaloid fraction which was converted to the base by washing with 5% ammonium hydroxide. The benzene solution was concentrated to dryness *in vacuo* to yield 8.7 g. of a crude aricin-rich fraction, A.

The aqueous phase remaining after the benzene extraction was adjusted to *p*H 10.5, and extracted with four 750-ml. portions of chloroform to yield 18.8 g. of a crude ajmaline-rich fraction, B. The chloroform insoluble precipitate remaining after the extraction of fraction B weighed 18 g., and still contained alkaloids, as indicated by a positive Mayer's test.

Separation of Aricin, Ajmaline, Reserpine and Tetrahydroalstonine.—The crude aricin-rich alkaloid fraction A, 8.7 g., was dissolved in a minimum of hot methanol, and allowed to cool overnight. Crystalline aricin, 3.45 g., m.p. 178–183°, separated. An additional 0.75 g. of somewhat cruder product was recovered on concentration of the mother liquors, to yield a total of 4.20 g. (1.0%) of crude crystalline product. A portion was purified by recrystallization from methanol to yield pure aricin, m.p. 185–186°, mixed m.p. with an authentic sample not depressed. The infrared spectrum and R_f value on two papergram systems were identical to those of an authentic sample.

The mother liquors from the crystallization of the aricin were concentrated to dryness, dissolved in 10 ml. of glacial acetic acid and diluted to 100 ml. with water. A crop of crude crystalline alkaloid acetate, 1.05 g., separated overnight. Paper chromatographic examination showed it to be predominantly aricin, with some tetrahydroalstonine (see below).

The remaining solution was extracted with five 100-ml. portions of benzene, the combined benzene extracts washed with 5% ammonium hydroxide, and concentrated to dryness to yield 1.10 g. of mixed aricin-depleted alkaloids. A portion, 0.24 g., of this material was redissolved in 15 ml. of benzene, and chromatographed on a column containing 35 ml. of Merck acid-washed alumina. Benzene, plus increasing increments of methanol, was used as the eluent. Results are shown in Table I.

Fraction 2 was purified by repeated recrystallization from methanol to yield 20 mg. of tetrahydroalstonine, m.p. 230–232°, $[\alpha]^{25}D$ −98° (*c* 0.2, $CHCl_3$) mixed m.p. with an authentic sample[6] not depressed. Comparison of the infrared spectra, and R_f value with those of authentic tetrahydroalstonine confirmed the identity. Substantial additional quantities of tetrahydroalstonine were shown to be present in the crude aricin acetate crop isolated before chromatography. Fraction 3 yielded traces of additional crystalline aricin, while fraction 4 yielded crystalline ajmalicine, m.p. 260–263° (*in vacuo*), further identified by comparison of infrared spectrum and R_f values with those of an authentic sample. Fractions 7 and 8 were recrystallized from meth-

(5) E. Schlittler, H. Schwartz and F. E. Bader, *Helv. Chim. Acta*, **35**, 271 (1952).

(6) The authentic tetrahydroalstonine was prepared from alstonine by the method of R. C. Elderfield and A. P. Gray, *J. Org. Chem.*, **16**, 506 (1951).