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 pH of the effluent and the influent are the same to within ± 0.02 pH unit before the sample (1 to 2 ml. adjusted to pH 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*

BY F. A. HOCHSTEIN

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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 pH 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).

TABLE I

Fraction	Methanol in solvent, %	Volume of solvent, ml.	Wt. solid eluted, mg.	Alkaloids present[a]
1	0	300	0	None
2	.5	250	50	Tetrahydroalstonine
3	.5	220	60	Tetrahydroalstonine + aricin
4	1.0	420	50	Ajmalicine
5	2.0	150	5	Unidentified alkaloid A
6	3.5	270	15	Reserpine
7	5	100		Reserpine
8	25	100	35	Reserpine
9	100	150	20	Unidentified alkaloid B

[a] As indicated by chromatography with benzene-cyclohexane solvent on formamide-impregnated paper.

anol to yield 20 mg. (0.002%) of reserpine, m.p. 265–266°, mixed m.p. with an authentic sample not depressed. The infrared spectrum and R_f values confirmed the identity of this material. A quantitative assay on fractions 7 and 8 indicated the presence of approximately 0.004% reserpine.

Ajmaline.—Fraction B from the original alkaloid separation was dissolved in a minimum of hot methanol, and held in the refrigerator overnight. Colorless crystals of ajmaline, 3.5 g., m.p. 148–152°, separated, and were removed by filtration. An additional 2.1 g. (total yield, 1.35%) of crystalline ajmaline were obtained on concentrating the mother liquors. A portion of this material was purified by recrystallization from aqueous methanol to yield pure ajmaline, m.p. 158–160° dec. The identity of the product was confirmed by comparison of the infrared spectrum and R_f values with those of an authentic sample.

The ajmaline mother liquors, 12 g. of dry weight, appeared from paper chromatography to contain in addition to substantial additional amounts of ajmaline, an unidentified weakly basic alkaloid, and a strong base similar in behavior to alstonine. However, alstonine was not isolated in pure form.

Acknowledgment.—We are indebted to Mrs. A. Paradies for her very capable assistance with this problem, and to Mr. W. H. Boegemann for the paper chromatographic studies, We should like to express our thanks to Dr. R. C. Elderfield for a generous sample of pure alstonine.

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The Structure of Frankincense Gum

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Frankincense gum from *Boswellia carterii* like myrrh gum[3,4] is an oleogum resin which contains some polysaccharide material. We report some properties of this carbohydrate portion. Owing to the small quantity of material available and because of large losses encountered in purifying it, this work is incomplete.

The gum was hydrolyzed with N sulfuric acid and the neutralized hydrolysate ($BaCO_3$) fractionated on cellulose in the usual way.[5]

D-Galactose and L-arabinose were thus isolated and identified. In addition two other substances, possibly fucose and rhamnose, were isolated, but in trace amounts only. The uronic acid was obtained as the barium salt and shown to be 4-*O*-methyl-D-glucuronic acid.

Although the sugars present in this gum are the same as those in gum myrrh[4] the molar ratios are quite different, *viz.*, galactose–arabinose–methylglucuronic acid, 7:1:4 for frankincense gum as compared with 8:2:7 for gum myrrh. The specific rotations are also quite different (−8 and +32°, respectively). On the other hand, their equivalents are of the same order of magnitude (545 and 460, respectively).

Autohydrolysis of these two gums was carried out under comparable conditions, and the changes in the composition of the hydrolysates followed by spotting samples on paper at intervals of time. Chromatograms in each case were run in both basic and acidic baths. Arabinose appeared in the hydrolysates at about the same time (6–9 hours) in each case, galactose rather sooner in frankincense (*ca.* 30 hours) than in myrrh (*ca.* 50 hours). Aldobiuronic acid also appeared sooner in the former (*ca.* 50 hours) than in the latter (*ca.* 70 hours). No other oligosaccharide materials were detected on the chromatograms.

Chromatography of an hydrolysate (6 hours with N sulfuric acid) in solvent (a) for 72 hours revealed a spot in the same position as that for 6-*O*-β-(4-*O*-methyl-D-glucuronosyl)-D-galactose. This acid together with 4-*O*-α-(4-*O*-methyl-D-glucuronosyl)-D-galactose occurs in gum myrrh.[4] The apparent lack of a 1,4-linked aldobiuronic acid in frankincense gum indicates a fundamental difference between two polysaccharides, which otherwise have several features in common.

Experimental

Unless otherwise stated, concentration of solutions was carried out at 40° (40 mm.) and specific rotations were measured in aqueous solution. Paper chromatograms were run in (a) ethyl acetate–acetic acid–formic acid–water (18:3:1:4)[6] or (b) butanol–pyridine–water (9:2:2).

Isolation and Purification of the Polysaccharide.—The gum resin (44 g.) in the form of small yellow nodules was extracted with hot acetone; evaporation of the extract left a pale yellow oil (17 g., 39%). The residue was dissolved in water (500 cc.), heated to about 85° and a slurry of cadmium hydroxide from cadmium sulfate (10.8 g.) and N sodium hydroxide (87 cc.) was added with rapid stirring. The mixture was centrifuged and the clear liquid shaken with a mixture of chloroform (0.25 vol.) and butanol (0.1 vol.) for 1 hour and then centrifuged.[7] The chloroform–protein gel formed a thin layer between the clear aqueous and chloroform layers. The aqueous layer was decanted and the process repeated until no further gel formed (4 treatments). The aqueous solution was then passed through columns of IR-120 and IRA 400 resins, and the gum acid (4.8 g.) was isolated as a white material by precipitating it in ethanol. It was collected on the centrifuge, washed and then dried at 60° (0.2 mm.), $[\alpha]^{19}D$ $-8 \pm 1°$ (*c* 1.0). Found: N, 0.5; OMe, 5.4; sulfated ash 0.37%, equiv. (by titration), 560 (545, on ash and protein free basis). Chromatography of the neutralized ($BaCO_3$) hydrolysate of this material (hydrolyzed for 16 hours in N sulfuric acid at 100°) gave spots (with *p*-anisidine hydrochloride) corresponding to galactose, arabinose and a monomethyl uronic acid. Extremely faint spots were present corresponding to fucose and rhamnose.

A quantitative determination of the non-acidic reducing sugars, after separation in the normal way on a chromato-

(1) Queen's University, Kingston, Ontario, Canada.
(2) National Chemical Research Laboratory, South African Council for Scientific and Industrial Research, Pretoria, South Africa.

(3) L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.*, 796 (1952).
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(7) M. G. Sevag, D. B. Lackman and J. Smolens, *J. Biol. Chem.*, **124**, 425 (1938).