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Erythrina Alkaloids. XII. Chromatographic Analyses of Erysodine, Erysovine and "Erysocine" and Technique for Preparative Isolation[1]

BY KARL FOLKERS AND JOHN SHAVEL, JR.

The isolation and characterization of erysopine, erysodine, erysovine[2] and erysonine[3] from various species of *Erythrina* have been described. These four alkaloids were obtained by appropriate techniques subsequent to their liberation by the acid hydrolysis of their aqueous solutions after complete removal of the free alkaloids. Difficulties in isolating homogeneous alkaloids from species of *Erythrina* have been observed frequently in published[4] and unpublished studies. It is to be emphasized again that special care should be given to establishing the homogeneity of any new free *Erythrina* alkaloid. Chromatographic analyses have been applied to alkaloids,[5] and preliminary experiments on erysonine[3] confirmed the purity of samples obtained by crystallization technique. This paper describes the results of further chromatographic analyses on pure eryso- alkaloids as a further check on purity and application of such adsorption methods for actual preparative separation of *Erythrina* alkaloidal mixtures.

The melting point and specific rotation of standard samples of erysodine and erysovine were not altered by chromatographic analyses over aluminum oxide. Pure erysopine was not sufficiently soluble in the ordinary solvents to make this analysis feasible. It was soluble in morpholine, as was erysonine,[3] but it was unstable in this solvent, the solution becoming dark green in color, probably because of oxidation due to its two ortho phenolic hydroxyl groups.[2] It was strongly adsorbed and was badly decomposed after elution. However, it is not so essential to confirm the purity of erysopine or isolate it by this technique, since the normal solvent isolation and purification is dependable because of the low solubility of this alkaloid.

"Erysocine" was described[2] as an alkaloid of apparently constant melting point, specific rotation and elementary analyses as isolated from four species of *Erythrina*, and from six other species of *Erythrina* listed in Table II. Gentile and Labriola recently isolated[6] "erysocine," besides erysodine and erysopine, from *E. falcata* Benth. in Argentina. A standard sample of "erysocine" was found to be separated into approximately equal parts of erysodine and erysovine by analysis over alumina. Since these two substances have identical empirical formulas, $C_{18}H_{21}NO_3$, elementary analyses were not significant. "Erysocine," from *E. sandwicensis* Deg.,[2] *E. flabelliformis* Kearney,[2] and *E. costaricensis* Micheli,[3] and from the six species in Table II of this paper, was separated into the two components. Only erysovine was obtained from the "erysocine" of *E. Poeppigiana* (Walp.) O. F. Cook[2] because of the paucity of the sample. Thus, sufficient samples of "erysocine" have been resolved into erysodine and erysovine to show that it is not a single alkaloidal entity.

"Erysocine" might be a molecular complex of erysodine and erysovine obtainable from ether or ethanol, the solvents used for crystallization. Erysodine and erysovine have similar solubilities in ether and it would be expected that their separation in this solvent would be difficult. Conclusive proof as to whether "erysocine" is just a mixture of erysodine and erysovine, mixed crystals or a complex of the two, remains to be settled.[7] Alkaloidal complexes are not unknown; for example, the ergot alkaloidal product, erygoclavine, was shown[8] to be an equimolecular mixture of ergosine and ergosinine. Stoll[9] found sensibamine to be a similar complex of ergotamine and ergotaminine.

Because of the curare-like action of so many species of *Erythrina*,[10] it was of interest to charac-

(1) Presented in part before the Division of Organic Chemistry at the Meeting of the American Chemical Society in Atlantic City, N. J., September 10, 1941.

(2) Folkers and Koniuszy, THIS JOURNAL, **62**, 1677 (1940).

(3) Folkers, Shavel and Koniuszy, *ibid.*, **63**, 1544 (1941).

(4) Folkers and Koniuszy, *ibid.*, **61**, 1232 (1939); **62**, 436 (1940).

(5) Zechmeister and Cholnoky, "Principles and Practice of Chromatography," John Wiley and Sons, New York, N. Y., 1941, p. 233. Strain, "Chromatographic Adsorption Analyses," Interscience Publishers, Inc., New York, N. Y., 1942, p. 101; see also Ruzicka, Dalma and Scott, *Helv. Chim. Acta*, **24**, 63 (1941).

(6) Gentile and Labriola, *J. Org. Chem.*, **7**, 136 (1942).

(7) Preliminary determination of an equilibrium diagram, based on melting points in a capillary, indicated such a complex. Molecular weight determination of "erysocine" by the freezing point depression of dioxane showed negligible association in this solvent. Determination of freezing points of molten erysovine and "erysocine" for plotting cooling curves was not satisfactory because of the decomposition of the molten alkaloids.

(8) Smith and Timmis, *J. Chem. Soc.*, 396 (1937); Köfler, *Arch. Pharm.*, [276] **40**, 61 (1938).

(9) Stoll and Schweiz, *Med. Woch.*, **65**, 1077 (1935); Köfler *Arch. Pharm.*, **275**, 455 (1937).

(10) Folkers and Unna, *J. Am. Pharm. Assoc.*, **28**, 1019 (1939).

terize the liberated alkaloidal fraction of certain other species of the genus for possible isolation of new physiologically active alkaloids. The data on seven such species are in the Experimental Part and the alkaloids isolated are indicated in Table I by positive signs. Widespread occurrence of the liberated alkaloids, erysodine, erysovine and erysopine, is observed[2,3] and is in contrast to the more limited range of occurrence of the free *Erythrina* alkaloids such as erythraline or erythramine.

TABLE I

ISOLATION OF ALKALOIDS

Plant	Alkaloids Erysodine	Erysovine	Erysopine
E. cubensis Wright	+	+	+
E. pallida Britton & Rose	+	+	+
E. arborescens Roxb.	+	+	+
E. Folkersii Kruk.	+	+	
E. velutina Willd.	+	+	
E. excelsa Baker	+	+	+ (?)
E. Berteroana Urb.	+	+	

The ether extraction technique previously described[2] for the isolation and separation of erysovine and erysodine, which also resulted in "erysocine" for some species, has been abandoned in favor of the chromatographic technique. Actually, the isolation of pure erysovine in quantity was quite unsatisfactory, and the chromatographic method described herein has been a desirable improvement.

Experimental Part

Isolation of Alkaloids.—The data on the generalized part of the procedures have been recorded in Table II, Parts A and B. The details of the procedures were analogous to those of the isolations described in paper IX.[2] Reference to the General Remarks to the Experimental Part of Paper IX[2] and X[3] should also be made for additional information which is not repeated here. Those details which cannot be tabulated satisfactorily, concern the fractional crystallizations of the liberated alkaloids and their identification. These data are described briefly as expanded notes to Part B of Table II. For the hydrolyses, after removal of the free alkaloidal fraction, the aqueous solutions were acidified as described in note *c*. For exploratory work, successive hydrolyses have proved helpful for examination of the alkaloids because of the different rates of hydrolysis of the combined alkaloids, but for subsequent preparative work, single prolonged hydrolyses have been used. The numbers of the specimens were assigned by Mr. B. A. Krukoff to the botanical specimens taken from the same plants as the seeds.[11] These specimens are deposited with the New York Botanical Garden.

General Procedure for the Chromatographic Analyses.—For 100–300 mg. of alkaloid, a 15 × 1 cm. column of aluminum oxide Merck (according to Brockmann) was found to be satisfactory. For 1 g. of alkaloid, a 30 × 1.9 cm. column was used.

For chromatographing erysovine and "erysocine," chloroform was preferred as the initial solvent and developing agent. For erysodine, chloroform was preferred for the solution, but ethanol was a better developing solvent.

It was found that when ethanol, morpholine or water solutions were passed over alumina, fine particles of alumina were dispersed which were not removed from the eluate by filter paper. The solutions were cloudy and, on standing, slowly deposited a fine sediment of alumina. Consequently, such eluates were concentrated *in vacuo*, and the residue was dissolved in chloroform and passed through a 2–3 cm. column of alumina. A sufficient amount of chloroform was used to elute the alkaloids.

During development, the column was examined under ultraviolet light, and occasional fluorescent bands were marked with a crayon and followed. Erysodine exhibited a weak yellow-green fluorescence, whereas erysovine seemed to exhibit even less fluorescence. In general, these weak bands were of little help. At the top of the column, there was always a light to dark brown band of decomposition products which had a vivid green fluorescence under ultraviolet light. These top bands remained there during the development and were discarded when the alumina was taken out for elution.

When the amount of alkaloid being eluted by washing the column decreased to a small value it was desirable to elute the remaining material by other means. The method used has been to put the alumina in a glass thimble of a continuous extractor shown in Fig. 1, and extract for several hours with chloroform. This procedure consistently gave almost quantitative recovery of the residual alkaloid. When alkaloid samples were adsorbed so strongly that only very small eluated residues were obtained, the column of adsorbant was divided into several parts, each of which was eluted in the continuous extractor.[12]

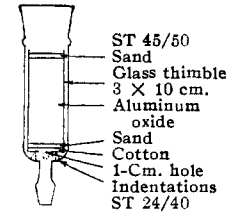

Fig. 1.[12]

Chromatographic Analysis of Erysodine.—The erysodine used was from *E. flabelliformis* Kearney (Jones 9485) and showed m. p. 200–201°, $[\alpha]^{25}D$ +251°, 75.8 mg./10 ml. ethanol, l = 1. The 3.66 g. was dissolved in 100 ml. of chloroform and passed into a 45 × 2.3 cm. column. There was a 5-mm. light brown top band which represented decomposition products. The tube under ultraviolet light showed a 5-mm. band of a light yellow-green fluorescence located 10 cm. from the top. The column was then developed with absolute ethanol. After collection of five fractions of the filtrate, the alumina was eluted with chloroform in the continuous extractor. The data are in Table III. Rotations were taken in ethanol at 30–60 mg./10 ml. concentration.

(11) Krukoff, *Brittonia*, **3**, 205 (1939); *Am. J. Botany*, **28**, 683 (1941).

(12) The sand at the bottom of the thimble prevented passage of the alumina, and the sand at the top prevented splattering of the adsorbent and its being washed down into the flask, causing "bumping." This extractor was used for a 30 × 1.9 cm. column of alumina.

TABLE II

DATA ON THE ISOLATION OF ALKALOIDS FROM SPECIES OF ERYTHRINA

PART A. FREE ALKALOIDAL FRACTION

Line	Plant	Collectors' names and specimen numbers	Amount seeds, g.	Fatty fraction, %	Alcohol[a] extractives, %	Free alkaloidal fraction,[b] %	Hypaphorine hydrochloride,[e] %
1	*E. cubensis* Wright	Acuna 9626	106.0	16.0	18.1	0.6	[f]
2	*E. cubensis* Wright	Acuna 9234	9.5	14.1	19.8	.7	..
3	*E. pallida* Britton & Rose	Wortley 9257	785.0	15.2	13.6	.41	..
4	*E. pallida* Britton & Rose	Wortley 9257	1030.0	11.7	14.5	.40	..
5	*E. pallida* Britton & Rose	Wortley 9257	705.0	13.0	15.3	.49	6.7
6	*E. arborescens* Roxb.	Ghose 9228	707.0	14.3	22.1	.27	[f]
7	*E. Folkersii* Kruk. & Mold.	Kinloch 9167	650.0	15.4	16.3	.07	[f]
8	*E. velutina* Willd.	Vasconcellos 9263 Sobrinho	650.0	9.2	20.0	.32	2.0
9	*E. velutina forma aurantiaca* (Ridl.) Kruk.	Rocha 9272	50.0	15.2	22.4	.25	..
10	*E. excelsa* Baker	Thomas 9342	347.0	9.6	25.6	.34[n]	2.4

PART B. LIBERATED ALKALOIDAL FRACTION

		Acid hydrolyses[c] First T.	First Y.	Second T.	Second Y.	Third T.	Third Y.	Total liberated alkaloidal fraction,[d] %	Alkaloids
1	*E. cubensis* Wright	10	0.78	75	2.05	90	0.06	2.89	[h]
4	*E. pallida* Britton & Rose	20	0.32	60	1.09	60	.50	1.96[j]	[i]
5	*E. pallida* Britton & Rose	10	1.28	60	0.95	60	.05	2.28	..
6	*E. arborescens* Roxb.	45	0.32	90	.49	120	.62	1.43	[k]
7	*E. Folkersii* Kruk. & Mold.	60	.22	120	.06			0.28	[l]
8	*E. velutina* Willd.	10	.93	20	.17	30	.20	1.30	[m]
9	*E. velutina forma aurantiaca* (Ridl.) Kruk.	15	1.2					1.2	..
10	*E. excelsa* Baker	15	1.01	60[g]	.85	60[g]	.54	2.40	[o]

[a] Methanol was used. [b] The free alkaloidal fraction was removed by the preferred procedure as described in paper IX. [c] The hydrolyses were made on aqueous solutions acidified with hydrochloric acid to about *p*H 2–2.3 except in a few cases where the solutions were acidified to *p*H 1 as indicated. T. = time in minutes, Y. = % yield of crude liberated bases after removal of the chloroform solvent. [d] The total yield of chloroform residues. [e] The hypaphorine was removed by the alternative procedure as described in paper IX. Further remarks on the isolation of hypaphorine are found in paper III. [f] Examination for hypaphorine was not made. [g] Hydrolysis was made at *p*H 1.

[h] **Erysovine, Erysodine and Erysopine from E. cubensis Wright.**—The 830 mg. of chloroform residue obtained from the first hydrolysis did not give a green color test with ferric chloride, showing the absence of erysopine. When treated with 1 ml. of ethanol, crystallization took place and 548 mg. of crystals, m. p. 154–157°, was obtained. Ether fractionation and recrystallization gave 223 mg. of "erysocine," m. p. 160–161°. A 162-mg. quantity of this fraction was dissolved in 5 ml. of chloroform and passed into a 1.5 × 1 cm. column. On developing with 40 ml. of chloroform, the first eluate gave 56 mg. of erysovine, which after crystallization from ether, showed m. p. 178–178.5°, $[\alpha]^{25}D$ +230°. Elution of the alumina in the extractor gave 108 mg. of alkaloid which gave 12 mg. of erysodine, after one recrystallization from ethanol, m. p. 200–201°, $[\alpha]^{25}D$ +248°. The second hydrolysis yielded 2.175 g. of alkaloids which gave a green color test with ferric chloride, showing the presence of erysopine. Treatment with 4 ml. of ethanol and refrigeration yielded 1.154 g. of m. p. 168–172°. When this material was heated with about 12 ml. of hot ethanol and filtered, 423 mg. of insoluble bases (A) of m. p. 197–198° was obtained. After refrigeration of the filtrate, 431 mg. of crystals (B) of m. p. 198–199° was obtained, which yielded 351 mg. of pure erysodine, m. p. 201–202°, $[\alpha]^{25}D$ +248°, after recrystallization from ethanol. When the crystals (A) were heated with about 4 ml. of hot ethanol and filtered, 51 mg. of crystals (C) of m. p. 214° was obtained. These were recrystallized twice from ethanol to yield 25 mg. of pure erysopine, m. p. 242–243°, $[\alpha]^{25}D$ +265.5° in 40% glycerol and 60% ethanol.

[i] **Erysovine and Erysodine from E. pallida Britton and Rose.**—The 3.271 g. of residue (negative green color test with ferric chloride) from the first hydrolysis was extracted with 300 ml. of boiling ether. There was 96 mg. of insoluble material of m. p. 143–146°. The ether solution yielded 2.245 g. of crystals of m. p. 146–150°. Three recrystallizations of this crop yielded 810 mg. of "erysocine," m. p. 161–162°. A 544-mg. quantity of "erysocine" was dissolved in 50 ml. of chloroform and passed into a 30 × 1.9 cm. column. On developing with 100 ml. of chloroform, the first eluate yielded 431 mg. of gum which, after two recrystallizations from ether, gave 123 mg. of pure erysovine showing m. p. 178–179°, $[\alpha]^{25}D$ +234°. The remaining erysodine adsorbed on the alumina was not sought. The 11.237 g. of residue from the second hydrolysis was combined with the 5.186 g. of residue from the third hydrolysis. Only the latter material gave a slight green color test with ferric chloride for erysopine. The com-

bined bases were extracted with 800 ml. of boiling ether. There was 3.206 g. of insoluble bases of m. p. 193–195°, and the ether extract yielded 4.476 g. of m. p. 157–159°, which corresponded to the material chromatographed before. The insoluble bases were recrystallized twice from ethanol to give 1.5916 g. of erysodine of m. p. 198–199° and $[\alpha]^{25}D$ +250°.

[j] **Erysopine from E. pallida Britton and Rose.**—A fourth hydrolysis of sixty minutes yielded 309 mg. of solid bases, which gave a positive color test with ferric chloride for erysopine. Treatment with 1 ml. of ethanol yielded 131 mg. of crystals of m. p. 186–188°. Five recrystallizations from ethanol gave 8 mg. of erysopine, m. p. 242–243°, $[\alpha]^{25}D$ +262.5°, in 40% glycerol and 60% ethanol. A fifth hydrolysis for sixty minutes at *p*H 1 yielded only 259 mg. of brown semi-crystalline residue which gave a positive color test for erysopine.

[k] **Erysovine, Erysodine, and Erysopine from E. arborescens Roxb.**—The 2.227 g. of bases from the first hydrolysis was triturated with 3 ml. of ethanol at 25° and the insoluble portion, 994 mg., m. p. 160–178°, was extracted with 50 ml. of boiling ether. The insoluble portion (A) was 311 mg., m. p. 164–190°, and the filtrate was concentrated to give a second crop (B), 413 mg., m. p. 163–164°. The portion (B) was recrystallized from ether to give 335 mg. of "erysocine," m. p. 162–163°. A quantity of 327 mg. of this fraction was then dissolved in 5 ml. of chloroform and passed into a 15 × 1 cm. column. On developing with 15 ml. of chloroform, the first eluate gave 139 mg. of erysovine, which after one recrystallization from ether showed m. p. 178–178.5°, $[\alpha]^{25}D$ +235°. The second eluate was added to the extract from the continuous elution of the adsorbent and, after distillation, it gave 184 mg. of residue which after one recrystallization from ethanol gave 35 mg. of pure erysodine, m. p. 199–200°, $[\alpha]^{25}D$ +250°. The portion (A) was triturated twice on the filter with ethanol and the insoluble portion left was 197 mg., m. p. 164–195–197°. This crop was recrystallized from ethanol to give 116 mg. of erysodine, m. p. 199–200°, $[\alpha]^{25}D$ +249°.

The second hydrolysis gave 3.429 g. of bases, which, after trituration with 5 ml. of ethanol, gave 1.367 g. of erysodine, m. p. (and mixed) 201–203°. The second crop was fairly pure erysodine, m. p. 197–200°. During the chloroform extraction after the third hydrolysis, 1.466 g. of erysopine separated from the aqueous solution and was filtered, m. p. (and mixed) 240–242°. Recrystallization from ethanol gave pure erysopine, m. p. 240–242°, $[\alpha]^{25}D$ +264°. The chloroform extraction residue amounted to 2.948 g. and yielded 1.105 g. of erysodine by crystallization.

[l] **Erysovine and Erysodine from E. Folkersii Kruk. and Mold.**—The 1.412 g. of bases from the first hydrolysis did not give a green color test with ferric chloride. It was triturated with 1 ml. of ethanol and the insoluble portion was 684 mg. (A). The filtrate was combined with the similar filtrate from the second hydrolysis, and after concentration the 811 mg. of residue was dissolved in aqueous sodium hydroxide solution and extracted six times with chloroform. The 402 mg. of solvent residue was triturated with 0.4 ml. of ethanol to give 127 mg. of insoluble material, m. p. 159–161° (B). The mother liquor on standing yielded large crystals which were fairly pure erysovine, m. p. 173.5–175°. The insoluble portion (B) was recrystallized once from ethanol and once from ether to give 23 mg. of "erysocine," m. p. 161–162°. A 15-mg. portion of this complex was dissolved in 1 ml. of chloroform and passed into a 3 × 1 cm. column to give 3 mg. of base, m. p. 160–161°. The second development gave 5 mg., m. p. 161–162° (clear at 178°) and on recrystallization from ether yielded 2 mg. of erysovine, m. p. 175–176° (clear, 180°, indicating presence of some erysodine). Elution of the alumina gave 5 mg. of base, m. p. 170–174° (clear at 190°). On recrystallization from ethanol, it yielded 2 mg. of erysodine, m. p. 197–198°, $[\alpha]^{25}D$ +244°. The insoluble portion (A) was recrystallized from ethanol to give 438 mg. of pure erysodine, m. p. (and mixed) 202–203°. The second hydrolysis gave 395 mg. of bases which was triturated with 0.4 ml. of ethanol to give 103 mg. of solid of m. p. 199–201°. After two recrystallizations from ethanol, pure erysodine was obtained, 10 mg., m. p. 200–201°, $[\alpha]^{25}D$ +249°.

[m] **Erysovine and Erysodine from E. velutina Willd.**—The 6.039 g. of bases from the first hydrolysis gave a negative green color test with ferric chloride. Trituration with ethanol, and subsequent recrystallization from ether of the solvent insoluble residue yielded 1.991 g. of "erysocine," m. p. 162–163°. A 1.108-g. quantity of this substance was dissolved in 25 ml. of chloroform and passed into a 30 × 1.9 cm. column. On developing with 400 ml. of chloroform, the first eluate yielded 377 mg. of gum which on recrystallization from ether gave 277 mg. of pure erysovine, m. p. 178–178.5°, $[\alpha]^{25}D$ +230°. Elution of the adsorbent in the extractor yielded 716 mg. of residue which after one recrystallization from ethanol gave pure erysodine, m. p. 200–201°, $[\alpha]^{25}D$ +247°.

The second hydrolysis gave 1.152 g. and the third gave 1.295 g. of bases. Neither product gave the green color test with ferric chloride. Both gave crude erysodine on trituration with ethanol, which, on combination and recrystallization, gave 564 mg. of pure erysodine, m. p. 200–201°, $[\alpha]^{25}D$ +249°.

[n] **Erysodine from the Free Alkaloidal Fraction of E. excelsa Baker.**—The 1.184 g. of the free alkaloidal fraction gave 474 mg. of crude erysodine, m. p. 196–200°, after trituration with 1 ml. of ethanol. Three recrystallizations gave pure erysodine, m. p. 201–202°, $[\alpha]^{25}D$ +245°.

[o] **Erysodine and Erysovine from E. excelsa Baker.**—The 2.033 g. of bases, m. p. 148–150°, from the first hydrolysis was extracted with 50 ml. of boiling ether. There was 460 mg. of insoluble bases (A), m. p. 174–194°, and the filtrate yielded 569 mg. of bases (B), m. p. 158–175°, after concentration. Three recrystallizations of (A) from ethanol yielded 161 mg. of pure erysodine, m. p. 201–202.5°, $[\alpha]^{25}D$ +246°. Recrystallization of (B) from ether gave 403 mg. of "erysocine," m. p. 161–162°. This was dissolved in 5 ml. of chloroform and passed into a 15 × 1 cm. column. It was developed with 25 ml. of chloroform. There was obtained 43 mg. of erysovine, m. p. 178–178.5°, $[\alpha]^{25}D$ +235°, after one recrystallization from ether of the 94 mg. of residue. Elution of the alumina in the extractor yielded 67 mg. of erysodine which showed, after one recrystallization from ethanol, m. p. 200–201°, $[\alpha]^{25}D$ +250°.

The 1.760 g. of bases from the second hydrolysis was triturated with 2 ml. of ethanol, and the 785 mg. of insoluble bases, m. p. 197–200°, showed the presence of erysopine by the ferric chloride color test. Two recrystal-

lizations from ethanol gave pure erysodine, m. p. 201–202°, $[\alpha]^{25}D$ +246°. The third hydrolysis gave 1.084 g. of liquid bases which was deeply fluorescent in chloroform solution.

TABLE III

DATA ON ERYSODINE

Filtrate	Vol., ml.	Residue, g.	M. p., °C.	$[\alpha]^{25}D$
1	200	None		
2	100	1.119	199–200	+247
3	100	0.290	200–201	+243
4	200	.458	197–198	+252
5	200	.323	200–201	+245
Continuous eluate	(*ca.* 6 hr.)	.906	197–198	+245

The residues were not recrystallized before taking the constants. The eluates were slightly cloudy and on standing slowly deposited a slight, fine sediment of alumina; this might account for the slight variations in the constants of the fractions. This chromatographic analysis did not yield erysodine of significantly altered constants when compared to the starting material.

Chromatographic Analysis of Erysovine.—The specific rotation of erysovine has been redetermined on larger and purer samples and found to be $[\alpha]^{25}D$ +232–234° as compared to the original value, $[\alpha]^{25}D$ +252°.[2] The erysovine used was from *E. glauca* Willd.[2] (Wortley 9242) and showed m. p. 177–178°, $[\alpha]^{25}D$ +232°, 49.0 mg./10 ml. ethanol, $l = 1$. A quantity of 1.045 g. was dissolved in 20 ml. of chloroform and passed into a 30 × 1.9 cm. column. Fresh solvent was added and 50-ml. fractions of filtrate were collected. The data are in Table IV. The specific rotations were in ethanol at 10 to 28 mg./2 ml. concentration.

TABLE IV

DATA ON ERYSOVINE

Filtrate	Residue, mg.	M. p., °C.	$[\alpha]^{25}D$
1	None		
2	None		
3	83	177–178	+235
4	192	178–179	+232
5	142	178–179	
6	77	178–179	
7	55	176–177	
8	67	175–176	
Continuous eluate	423	178–179	+233

None of the fractions showed significantly different constants.

Chromatographic Resolution of "Erysocine" into Erysodine and Erysovine.—When a chloroform solution of erysocine was passed into a column of alumina and developed with chloroform, the first eluate was found to contain pure erysovine. An intermediate eluate containing erysovine and erysodine followed, after which the erysodine was slowly eluted. To effect almost quantitative removal of erysodine, the alumina was extracted continuously with chloroform. After removal of the solvent recrystallization of the residue from ethanol gave pure erysodine.

When a solution of erysovine in chloroform was quickly concentrated to dryness *in vacuo* at 50–60°, the residue was an amorphous fluff which was easily soluble in ether. The addition of a little ether followed by warming, quickly dissolved the fluff, and pure erysovine started to crystallize after a minute or two. This procedure was found to be the most efficient for giving pure erysovine with the minimum amount of recrystallizations.

The details on the obtaining of erysodine and erysovine from "erysocine" are described in the footnotes to Table II.

Preparative Isolation of Erysovine by Chromatographic Technique.—The crude alkaloids obtained from the first hydrolysis of an extract from *E. Berteroana* Urb. (Armstrong 9304) on several recrystallizations from ethanol yielded "erysocine," m. p. 159–161°, indicating the presence of erysovine and erysodine. Mr. Frank Koniuszy found that another similar extract yielded on hydrolysis two crops of alkaloidal mixtures. The first crop of 43 g., m. p. 169–172°, was dissolved in 150 ml. of chloroform and passed through a 92 × 4 cm. column, and was developed with chloroform. The data are in Table V. The weight of crystals represents the yield obtained after the gum remaining, after distillation of the chloroform eluant, was recrystallized from ether. The second crop of 24 g., m. p. 177–178°, was treated similarly and pure erysovine obtained.

TABLE V

ISOLATION OF ERYSOVINE

Filtrate	Volume, ml.	Crystals, g.	M. p., °C.	$[\alpha]^{25}D$
1	500	14	178	+236.9
2	1000	12	178	+236.7
3	2000	4.5	178	

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Summary

Chromatographic analyses of standard samples did not significantly alter the constants of erysodine and erysovine. All samples of "erysocine" were chromatographically resolved into approximately equal parts of erysodine and erysovine. The chromatographic technique is more satisfactory for the isolation and separation of pure erysovine from erysodine than the previously used ether extraction process.

Erysodine and erysovine have been isolated for the first time from *E. cubensis* Wright, *E. pallida* Britton and Rose, *E. arborescens* Roxb., *E. Folkersii* Kruk. and Mold., *E. velutina* Willd. and *E. excelsa* Baker. Erysopine was isolated anew from the first three mentioned species, and erysodine was isolated from *E. Berteroana* Urb. These three eryso- alkaloids have a very wide distribution in the seeds of species of the genus *Erythrina*.

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