and then extracted with ether. After washing the ether extracts until the washings were neutral to congo red, the ether was distilled. The residue obtained was dissolved in four times its volume of alcohol. One-fourth of this solution was neutralized with 5% sodium hydroxide and then the remainder of the alcoholic solution was added. After standing for several days the crystalline acid sodium salt was filtered and recrystallized from 30 cc. of alcohol. The *l*-abietic acid was freed by addition of dilute hydrochloric acid and subsequent recrystallization from dilute alcohol.

Source of acids used	Yield of *l*-abietic acid, g.	$[\alpha]^{20}D$	M. p., °C.
P. palustris oleoresin	2.3	−99°	163–166
P. palustris rosin	2.4	−97°	164–167
P. caribaea oleoresin	2.0	−97°	164–167
P. caribaea rosin	2.1	−98°	167–169

No lowering of the melting point was produced when these samples of *l*-abietic acid were mixed with *l*-abietic acid prepared by the method of Palkin and Harris.[6]

The Action of Sulfuric Acid on *l*-Abietic Acid.—Fifteen grams of *l*-abietic acid,[6] $[\alpha]^{20}D$ −104°, was added to cold concentrated sulfuric acid as outlined above. When separated into acid and neutral fractions, there was isolated only a trace of partially crystalline neutral material. The crude acid fraction was recrystallized from dilute alcohol and dried in vacuum at room temperature. The yield was 12.5 g. of material that melted at 169–172°, $[\alpha]^{20}D$ −95°, and showed no lowering of melting point when mixed with starting material.

Isomerization of *l*-Pimaric Acid with Concentrated Sulfuric Acid.—Four grams of powdered *l*-pimaric acid (m. p. 143–148°; $[\alpha]^{20}D$ −271°) was added during the course of ten minutes to 20 cc. of concentrated sulfuric acid, and cooled to −5 to −10°. Stirring and cooling was continued for forty-five minutes and then the light yellow colored solution was poured into 100 g. of ice. The precipitate was worked up as outlined above into neutral and acid fractions. There was only a trace of partially crystalline neutral material. Crystallization of the acid fraction from dilute alcohol gave 3.1 g. of material which melted at 170–172°; $[\alpha]^{20}D$ −94°. This material was converted into the acid sodium salt, recrystallized from alcohol, and isolated as the free acid, as outlined above. The dried material rotated $[\alpha]^{20}D$ −104°. Further purification was attempted by reconversion into the acid sodium salt and recrystallizing the salt twice more from alcohol. When the free acid was regenerated and dried in high vacuum at room temperature, it melted at 169–172°, $[\alpha]^{20}D$ −104°. When mixed with *l*-abietic acid prepared by the method of Palkin and Harris,[6] no lowering of the melting point took place. *Anal.* Calcd. for $C_{20}H_{30}O_2$: C, 79.41; H, 10.01. Found: C, 79.17; H, 10.18.

Lactonization of the Dihydroabietic Acid in Commercial Hydrogenated Rosin.—The acids were freed from neutral material, by extraction, as outlined above.

Twenty grams of the acids obtained from a commercial hydrogenated rosin was subjected to the action of cold concentrated sulfuric acid as outlined above. When separated into acid and neutral fractions, 7.7 g. of neutral crystalline material was obtained. After two recrystallizations from methyl alcohol, 3.2 g. of lactonized dihydroabietic acid, m. p. 131–132°, $[\alpha]^{20}D$ −4°, was obtained. This material showed no depression of melting point when mixed with authentic lactonized dihydroabietic acid.

Summary

Conversion to the isomeric lactone affords an excellent means for detecting dihydroabietic acid in the presence of large proportions of other resin acids.

By this means dihydroabietic acid has been shown to be present in the oleoresin and the rosin of *P. palustris* and *P. caribaea*.

Cold concentrated sulfuric acid has been shown to isomerize *l*-pimaric acid into *l*-abietic acid.

Lactonized dihydroabietic acid may readily be prepared from commercial hydrogenated rosin.

WASHINGTON, D. C. RECEIVED MARCH 14, 1939

[CONTRIBUTION FROM THE RESEARCH LABORATORY OF MERCK & CO., INC.]

Erythrina Alkaloids. III. Isolation and Characterization of a New Alkaloid, Erythramine

BY KARL FOLKERS AND FRANK KONIUSZY

From the studies which are being made in this Laboratory on species of *Erythrina*, a preliminary paper on twenty-six species showed that the principles of curare-like action were widely distributed in the genus, both geographically and in the various sections of the genus.[1] This wide distribution has been proven further by continued study of many additional species. The isolation of the first of several new physiologically active alkaloids was announced[2] recently and the details as well as new data will be submitted for publication soon.

The present paper describes the isolation and characterizes another of the physiologically active alkaloids, which has been named erythramine. It was isolated from the seeds of *Erythrina sandwicensis* Deg. and *Erythrina subumbrans* (Hassk.) Merrill. The identity of the *Erythrina* seeds

(1) Folkers and Unna, *J. Am. Pharm. Assoc.*, **27**, 689 (1938).

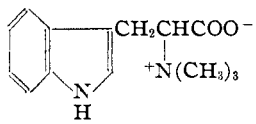
(2) Folkers and Major, THIS JOURNAL, **59**, 1580 (1937).

used in these studies was established either by accompanying herbarium material, which was determined by Mr. B. A. Krukoff at the New York Botanical Garden, or by other evidence which left no doubt regarding the identity.

Although it was claimed by Greshoff[3-5] that the seeds of *Erythrina subumbrans* contained a poisonous alkaloid, the nature of the substance was entirely unknown.

All of the results of analyses made on the free base, and the hydrochloride, hydrobromide, and hydriodide were in agreement with the empirical formula $C_{18}H_{21}NO_3$ for erythramine. The crystalline erythramine base was not stable upon standing, and was kept best in a black vial, and stored *in vacuo* at dry-ice temperature. Its hydrohalide salts were stable. Progress has been made toward the constitution of erythramine, and the results will be published later.

Hypaphorine was isolated for the first time from *Erythrina sandwicensis* and was re-isolated from *Erythrina subumbrans*. Hypaphorine was discovered by Greshoff[3,6] in seeds of *Erythrina subumbrans*, and was isolated from *Erythrina variegata* L. var. *orientalis* (L.) Merrill by Maranon and Santos.[7] It has been isolated anew in this Laboratory from many other species of *Erythrina*, and indeed seems to occur very widespread in the genus. Hypaphorine was shown to be I, the betaine of tryptophan.[8,9] The recorded yields of hypaphorine from the species herein



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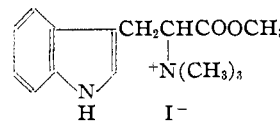

II

listed are not quantitative, since the chloride is moderately soluble in water, and the filtered amount depends on the volume of the concentrate.

It is not to be inferred that erythramine is the sole alkaloid present in the crude erythramine fraction as listed in Table I. However, it is considered the principal constituent. Examination of mother liquors on a much larger scale undoubtedly will yield minor alkaloids, and indeed this has been realized on another species of *Erythrina* containing erythramine.

Certain other species of *Erythrina* have been found to contain erythramine, but in these species it is associated with another alkaloid of very similar properties. Because these two mixed alkaloidal bases, and certain of their salts, have melting points that are only slightly depressed, the difficulties of manipulating these mixtures must be described in a separate paper. The identification of a hydriodide, isolated from another species of *Erythrina*, as that of erythramine, is best established by complete data on melting points, optical rotation, analytical work, and other derivatives, if possible. In this connection, the relative ease of isolating pure erythramine from *Erythrina sandwicensis* and *Erythrina subumbrans* is of interest in connection with the botany of these two species. *Erythrina sandwicensis* is endemic and is the only species in the Hawaiian Islands. *Erythrina subumbrans* comprises a species group occupying an isolated position in the entire genus.

Erythramine also was isolated from the crude chloroform residue from *Erythrina sandwicensis* as the hydrobromide, but the yields were poorer, and the hydrobromide possessed certain instabilities and solubilities which made it undesirable for purification purposes. The hydriodide was preferred.

Dr. Klaus Unna of the Merck Institute of Therapeutic Research has made a preliminary test on the activity of this alkaloid. The threshold dose of erythramine hydrobromide for curare-like paralyzation in frogs was 10 mg./kg., injected intralymphatically.

Hypaphorine has no paralyzing action on the frog, but produced a hyperexcitability[1] instead. However, it was expected that the methyl α-dimethylamino-β-(3-indole)-propionate methiodide, II, would be active for curare-like paralysis, and the experimental findings of the Merck Institute were in accord with this since the threshold dose for curare-like action was found to be 100 mg./kg. frog. The toxicity of this derivative in mice was 450 mg./kg. for the 50% lethal dose.

Experimental Part

Isolation of Erythramine and Hypaphorine

Erythrina sandwicensis **Deg. (L. W. Bryan 9160).**—A quantity of 200 g. of seed powder (40 mesh) was extracted in a Soxhlet for six hours with 750 ml. of petroleum ether. The yield of the yellow fatty oil, obtained after solvent re-

(3) Greshoff, *Mededeelingen uit's Lands. Plantentuin*, **7**, 29 (1890).
(4) Greshoff, *Ber.*, **23**, 3540 (1890).
(5) Greshoff, *Ber. deut. pharm. Ges.*, **9**, 215 (1899).
(6) Greshoff, *Mededeelingen uit's Lands. Plantentuin*, **25**, 54 (1898).
(7) Maranon and Santos, *Philippine J. Sci.*, **48**, 563 (1932).
(8) Van Romburgh, *Akad. Wetensch. Amsterdam. Wisk. Natk. Afd.*, **18**, 1177 (1911).
(9) Van Romburgh and Barger, *J. Chem. Soc.*, **99**, 2068 (1911).

moval, was 29.9 g. (14.9%). The seed powder was then extracted thoroughly with 750 ml. of methanol for thirty-five hours. The extract was concentrated at 30° and 18 mm. to a gum, and finally pumped out for three hours at 30° and 2 mm. There was 38.7 g. (19.3%) of residue which was dissolved in 200 ml. of water, made just acid with concentrated hydrochloric acid, and clarified by one extraction with 100 ml. of petroleum ether and two extractions with 100-ml. portions of chloroform. The aqueous solution, now freed of residual fatty oil droplets, was immediately made just alkaline with sodium bicarbonate, and extracted ten times with small portions of chloroform. The solvent was distilled at 30° *in vacuo*, and the residue was pumped out for two hours at 30° and 2 mm. The yield of the crude erythramine fraction was 736.4 mg. (0.37%).

The alkaline solution was acidified with hydrochloric acid and concentrated at 30° *in vacuo* to 75 ml. After standing for fifteen hours at 10°, the white needles of hypaphorine hydrochloride were filtered; yield, 1.96 g. (0.98%), m. p. 229–230°. The mixed melting point showed no depression. The actual yield of hypaphorine was greater, of course, because of the amount of the moderately soluble chloride remaining in solution. In other extractions of this same sample of seeds, where a lower concentration of the solution resulted, a yield of 1.54% was obtained.

Erythramine Hydriodide.—Erythramine was best isolated and purified from the crude alkaloidal fraction as the hydriodide. Thus, the 736 mg. was dissolved in 2 ml. of absolute ethanol and treated with the estimated quantity of sodium iodide (36.9 mg.) and glacial acetic acid. The amount and degree of dryness of the ethanol was adjusted so that the sodium acetate remained dissolved. It was distinguished readily by appearance (white) from the crystallizing hydriodide (yellow). After fifteen hours at 10°, there were filtered 335 mg. of crude iodide, m. p. 244° with decomposition. A second crop of 211.8 mg. with the same decomposition point was obtained by addition of a few drops of anhydrous ether to the mother liquor. Two recrystallizations from absolute ethanol gave yellow-orange needles of the constant melting point 249° with decomposition, $[\alpha]^{25}D$ +220°, C = 0.500, water.

Anal. Calcd. for $C_{18}H_{21}NO_3 \cdot HI$: C, 50.60; H, 5.15; N, 3.28. Found: C, 50.66, 50.71; H, 5.13, 4.90; N, 3.23.

Erythramine Hydrobromide.—Pure hydriodide (397 mg.) was dissolved in 10 ml. of water and made alkaline with sodium bicarbonate, whereupon a white turbidity appeared. After ten extractions with chloroform, solvent removal, and pumping out at 2 mm., there was obtained 310.4 mg. (88%) of erythramine base as a gum. This was dissolved in 1 ml. of absolute ethanol, treated with the calculated amount of 40% hydrobromic acid, and allowed to stand at 10°. The erythramine hydrobromide needles, recrystallized from ethanol, showed m. p. 228°, $[\alpha]^{26}D$ +203.2°, C = 0.500, water.

Anal. Calcd. for $C_{18}H_{21}NO_3 \cdot HBr$: C, 56.84; H, 5.78; N, 3.68; Br, 21.05. Found: C, 56.63, 57.09; H, 5.58, 5.48; N, 3.69; Br, 20.84.

Erythramine Hydrochloride Hemihydrate.—Pure erythramine base (68 mg. obtained from the pure hydriodide as described above) was dissolved in 5 ml. of absolute ether and treated with the required hydrogen chloride in absolute ethanol. The white precipitate was filtered and recrystallized from ethanol (very soluble) in a centrifuge tube. The erythramine hydrochloride hemihydrate was dried at 25° and 2 mm. for one hour before analysis; m. p. 249°.

Anal. Calcd. for $C_{18}H_{21}NO_3 \cdot HCl \cdot {}^1/_2H_2O$: C, 62.69; H, 6.72. Found: C, 62.84; H, 6.23.

Erythramine Hydrochloride Anhydrous.—The hydrochloride hemihydrate was dried at 140° and 2 mm. for five hours. It now showed m. p. 250° with decomposition.

Anal. Calcd. for $C_{18}H_{21}NO_3 \cdot HCl$: C, 64.36; H, 6.60. Found: C, 64.42, 64.06; H, 6.39, 6.30.

Erythramine Base.—An amount of 486 mg. of pure erythramine hydriodide from *Erythrina sandwicensis* (L. W. Bryan 9160) was converted to the free base by the treatment described. The yield was 431 mg. (99.5%). The colorless gum was distilled at 125° and 3.9×10^{-4} mm. in a molecular still. The condensate on the ceiling was very viscous.

Anal. Calcd. for $C_{18}H_{21}NO_3$: C, 72.21; H, 7.06. Found: C, 72.26; H, 6.82.

The high vacuum distillation had to be conducted properly (minimum heat and maximum vacuum in minimum time) or else complete decomposition of the alkaloid would ensue.

Erythramine base was very soluble in ethanol, methanol, benzene, and ethyl acetate. It was moderately soluble in diethyl ether and almost insoluble in petroleum ether.

Erythramine was recrystallized by the following technique. A sample of 517 mg. of base, which had been freshly obtained from analytically pure hydriodide from *Erythrina sandwicensis* (L. W. Bryan 9160), was treated quickly with 25 ml. of boiling diethyl ether. The dark, decomposed, and insoluble portion, which quickly formed, was removed by filtration. The ethereal solution was diluted with 15 ml. of petroleum ether and the solvents were then quickly distilled until the residual volume was about 5 ml. The flask was immediately placed in ice, and the inside was scratched with a spatula. Tiny white crystals soon formed; m. p. 104–105°. Carbon analyses on these crystals were 1–1.5% low because of solvent of crystallization which did not appear to be present in stoichiometric proportion. Drying at 61° and 2 mm. for one hour yielded pure erythramine; m. p. 103–104°; $[\alpha]^{29.5}D$ +227.6°, C = 0.188, ethanol.

Anal. Calcd. for $C_{18}H_{21}NO_3$: C, 72.21; H, 7.07. Found: C, 72.49; H, 6.88.

The crystalline base quickly became yellow upon standing. It was stored in a vial at 2 mm. pressure and at dry-ice temperature.

Isolation of Erythramine from Other Species of Erythrina.—The technique of isolation and purification of erythramine as the hydriodide has been described in detail for *Erythrina sandwicensis*. Erythramine has been isolated also from *Erythrina subumbrans* by the same general technique, and the significant data are summarized in Table I.

Identification of Hypaphorine.—The data concerning hypaphorine have been examined carefully, partly because of the frequency with which this alkaloid has been en-

TABLE I

DATA ON THE ISOLATION OF ERYTHRAMINE FROM SPECIES OF *Erythrina*

Plant	Collectors' names, specimens numbers and source	Amount seeds, g.	Fatty fraction	Alcohol residue, %	Hypaphorine hydrochloride, %	Crude erythramine fraction,[a] %	Authenticity of erythramine
E. sandwicensis Deg.	L. W. Bryan 9160 (Hawaiian Isl.)	200.0	14.9	19.3[e]	0.98	0.37[b]	Expt. part
E. subumbrans (Hassk.) Merrill	From Buitenzorg 9151 (Java)	20.3	12.6	22.2[d]	..	.40[c]	....
E. subumbrans (Hassk.) Merrill	Haigh 9171 (Ceylon)	200.0	12.7	23.0[d]	3.0	.25[c]	[f]
E. subumbrans (Hassk.) Merrill	Holttum 9204/34801 (Fed. Malay States)	685.0	14.1	22.7[e]	..	.32[b]	[g]

[a] It was essential that the crude erythramine fraction be extracted before the hypaphorine was removed exactly as described in the detailed procedure for *E. sandwicensis*. This is called the "preferred procedure," and the percentage figure which is obtained expresses very nearly the amount of the erythramine fraction which is actually present. The "alternative procedure" consisted of the separation of the hypaphorine before the extraction of the erythramine fraction. The "alternative procedure" gives a slightly increased percentage of the erythramine fraction, because of the introduction of other substances. [b] By the "preferred procedure," which expresses very nearly the true percentage. [c] By the "alternative procedure," which expresses a slightly increased percentage because of the presence of other substances. [d] Extracted with 95% ethanol. [e] Extracted with absolute methanol. This solvent is generally preferred because of greater extracting power. [f] Erythramine hydriodide, m. p. 248°; mixed m. p. 249°. *Anal.* Calcd. C, 50.60; H, 5.15. Found: C, 50.86; H, 4.92. $[\alpha]^{27}D$ +221.6°, $C = 0.296$, H_2O. [g] Erythramine hydriodide, m. p. 242°; $[\alpha]^{28}D$ +218.2°, $C = 0.2509$, H_2O. The melting or decomposition point varied somewhat with the rate of heating of the bath.

countered, and partly to check certain discrepancies in the literature.

Hypaphorine Hydrochloride.—The salt, as isolated from the aqueous concentrates derived from the species of *Erythrina* dealt with in this paper, generally showed the correct melting point or, if not, only one to three degrees low. Crystallized from water, it showed m. p. 231–232° with decomposition[10] and the odor of trimethylamine, $[\alpha]^{32}D$ +89.6°, $C = 0.502$, H_2O.

Anal. Calcd. for $C_{14}H_{19}N_2O_2Cl$: C, 59.33; H, 6.76; N, 9.89; Cl, 12.52. Found: C, 59.28, 59.40; H, 6.72, 6.76; N, 9.78; Cl, 12.13.

When 500 mg. of hypaphorine hydrochloride was dissolved in 10 ml. of 15% aqueous potassium hydroxide, and the solution refluxed for five hours, indole (m. p. 51–52°) was obtained by ether extraction, etc.

Hypaphorine Nitrate.—This salt showed m. p. 223.5–224.5° with decomposition.[11,12]

Hypaphorine.—The free betaine crystallized from one of the concentrated alcohol extracts and showed m. p. 236–237° with decomposition after crystallization from dilute alcohol; $[\alpha]^{27}D$ +113.1°; $C = 0.516$, H_2O.

Anal. Calcd. for $C_{14}H_{18}N_2O_2$: C, 68.25; H, 7.37; N, 11.38. Found: C, 68.13; H, 7.38; N, 11.22.

A sample did not lose any weight after drying at 100° (2 mm.), and the carbon–hydrogen analyses (C, 68.33; H, 7.33) and the m. p. (237°) were unchanged. There was no evidence of hydrate formation.[13]

Methyl α-Dimethylamino-β-(3-indole)-propionate Methiodide.—Hypaphorine hydrochloride (4 g.) was converted to the methiodide of methyl α-dimethylamino-β-(3-indole)-propionate by refluxing with 60 ml. of methanol, 6 ml. of methyl iodide, and 1.4 g. of sodium hydroxide for six hours; m. p. 200.5–201.5° with dec.[14]

Anal. Calcd. for $C_{15}H_{21}N_2O_2I$: C, 46.38; H, 5.45; N, 7.22. Found: C, 46.50; H, 5.43; N, 7.31.

Acknowledgments.—We are deeply grateful to Mr. B. A. Krukoff for his efforts which have made this study possible, for his advice on botanical matters, and for his constant interest. To those people who have contributed by obtaining the seed samples, we wish to express our sincere gratitude. The coöperation of Messrs. Douglas Hayman, Wilhelm Reiss, and W. B. Wright on microanalyses and other assistances has been greatly appreciated.

Summary

A new alkaloid, named erythramine, has been isolated from the seeds of *Erythrina sandwicensis* Deg. and *Erythrina subumbrans* (Hassk.) Merrill. Hypaphorine was isolated anew from *Erythrina sandwicensis* and was re-isolated from *Erythrina subumbrans*.

The crystalline erythramine base, hydriodide, hydrobromide, and the hydrochloride have been described. The microanalyses showed that erythramine has the empirical composition, $C_{18}H_{21}NO_3$.

Erythramine was strongly active in causing a curare-like paralysis in frogs.

Hypaphorine was converted to methyl α-dimethylamino-β-(3-indole)-propionate methiodide and this derivative was found to cause a curare-like paralysis in frogs when a high dose was administered.

RAHWAY, N. J. RECEIVED MARCH 9, 1939

(10) M. p. 227°, Maranon and Santos.[7]

(11) M. p. 215–220°, van Romburgh and Barger.

(12) M. p. 220°, Cahill and Jackson, *J. Biol. Chem.*, **126**, 29 (1938).

(13) A dihydrate was quoted in "Plant Alkaloids," by T. A. Henry, P. Blakiston's Son & Co., Philadelphia, 1924, 2d ed., p. 312; m. p. 238° with dec., Maranon and Santos;[7] m. p. 255; $[\alpha]D$ + 91–93°, Greshoff.[5,6] $[\alpha]D$ +94.7, van Romburgh and Barger.[9] We dissolved hypaphorine nitrate in water and added ammonia after the method of van Romburgh and Barger, and calculated on the basis of hypaphorine, found $[\alpha]D$ +113.8; m. p. 253–254°; $[\alpha]^{25}D$ +113.4, Cahill and Jackson.[12]

(14) M. p. 197°, van Romburgh and Barger.[9]