crystalline residue. The diamine was dissolved in 120 ml. of 4 *N* hydrochloric acid containing 4 ml. of 90% formic acid. The solution was refluxed for 40 minutes, cooled, and neutralized to *p*H 8 with 28% aqueous ammonia. The crystalline product was collected on a filter and washed with water. Recrystallization of the product from methanol–water (Darco was used) gave 5.5 g. of 1-(1′-D-sorbityl)-5,6-dichlorobenzimidazole, m.p. 198–200° with a transition at 147°. A sample recrystallized three times from methanol melted at 205–207° after a transition below 200°. After two additional crystallizations from acetic acid–water the melting point was 206–207° after a transition that began at 145°.

The 1-(1′-glycityl)-5,6-dichlorobenzimidazoles and 1-(1′-D-ribityl)-5,6-dimethylbenzimidazole are reported in Table I.

Summary

1-(1′-D-Ribityl)-5,6-dimethylbenzimidazole, 1-(1′-D-sorbityl)-5,6-dichlorobenzimidazole, 1-(1′-D-arabityl)-5,6-dichlorobenzimidazole and 1-(1′-D-xylityl)-5,6-dichlorobenzimidazole have been synthesized and have been found to be ineffective in producing regression of established lymphosarcoma implants in mice.

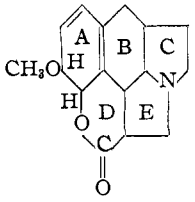
RAHWAY, NEW JERSEY RECEIVED JUNE 29, 1950

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

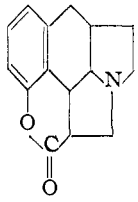
Erythrina Alkaloids. XX. Apo- and Isoapo-β-erythroidine

BY FRANK KONIUSZY AND KARL FOLKERS

Considerations of structure I for β-erythroidine and structure II for desmethoxy-β-erythroidine have been described.[1] β-Erythroidine was converted into desmethoxy-β-erythroidine by reaction with 30% sulfuric acid at 100°. The degradation product was isolated as a perchlorate. The reactions of β-erythroidine under other conditions of acid hydrolysis were also examined, and the results of these concomitant studies are summarized herein.



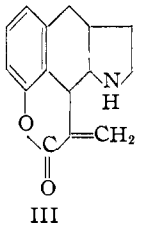
I



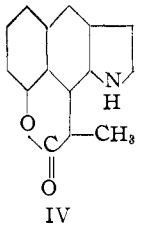
II

The reaction of β-erythroidine with concentrated hydrobromic acid at 100°, followed by fractional crystallization of the resulting free-base products, gave a crystalline compound, m. p. 144°, which has the composition $C_{15}H_{15}NO_2$. This product is isomeric with desmethoxy-β-erythroidine and was designated apo-β-erythroidine. It reacts with ferric chloride in aqueous solution to give an intensely purple-colored product; consequently, apo-β-erythroidine was used as the basis for the development of a colorimetric determination[2] of β-erythroidine.

The positive color reaction of apo-β-erythroidine with ferric chloride and the corresponding negative reaction with desmethoxy-β-erythroidine can be interpreted on the basis of a structural difference in the nitrogen-containing ring of apo-β-erythroidine. Although the hydrogenation of desmethoxy-β-erythroidine gave a hexahydro- derivative, the hydrogenation of apo-β-erythroidine has been found to give an octahydro- derivative. Octahydro-apo-β-erythroidine is a crystalline compound, m. p. 135–136°, which was also characterized as a crystalline hydrochloride. Thus, there is an additional double bond in apo-β-erythroidine, which differentiates it from the isomeric desmethoxy-β-erythroidine. Structure III may be envisaged for apo-β-erythroidine to account for its properties, and because the weak nitrogen–carbon bond in the >N—C—C—CO-grouping of structure I could be expected to be cleaved under appropriate conditions with either acid or alkali. Structure IV may be considered for the octahydro-apo-β-erythroidine.



III



IV

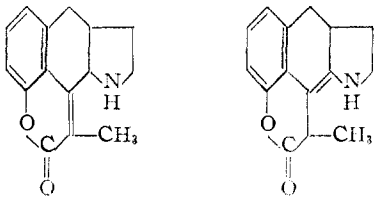
If apo-β-erythroidine has structure III, it would be expected that by suitable oxidation conditions, formic acid would be formed. The oxidation of apo-β-erythroidine with potassium permanganate in sulfuric acid solution did result in the production of formic acid. The formic acid was removed from the oxidation solution by distillation and was isolated as a *p*-bromophenacyl ester. This specimen of the derivative was identical with an authentic one.

It is evident from structure III that apo-β-erythroidine might also be prepared directly from desmethoxy-β-erythroidine, and that the latter compound may be an intermediate in the degradation of β-erythroidine to apo-β-erythroidine.

From the mother liquors of the crystallization of apo-β-erythroidine, there was obtained another crystalline compound, m. p. 154–155°, which also has the composition $C_{15}H_{15}NO_2$; this compound was designated isoapo-β-erythroidine. The hydrogenation of isoapo-β-erythroidine also resulted in the characterization of an octahydro- derivative, m. p. 134–136°, which is identical with octahydro-apo-β-erythroidine. If apo-β-erythroidine has an exocyclic bond as shown in structure III to account for the additional hydrogen absorption and degradation to formic acid, it is understandable that the exocyclic bond might shift, as in structure V,

(1) Koniuszy and Folkers, THIS JOURNAL, **72**, 5579 (1950).
(2) Dietz and Folkers, *J. Am. Pharm. Assoc.*, **35**, 48(1946).

so that it is conjugated with both the carbonyl and benzenoid groups. Accordingly, isoapo-β-erythroidine appears to have structure V (or possibly VI).



V VI

Oxidation of isoapo-β-erythroidine, under the same conditions applied to apo-β-erythroidine, did not yield formic acid; this result is consistent with structure V. However, a Kuhn–Roth determination on isoapo-β-erythroidine did yield acetic acid showing the presence of a carbon–methyl group in this compound. A Kuhn–Roth determination on apo-β-erythroidine also yielded acetic acid, but this result is quite probably due to the rearrangement of apo-β-erythroidine to isoapo-β-erythroidine under the conditions of acid and temperature used in this determination. A Kuhn–Roth determination on octahydro-apo-β-erythroidine also yielded acetic acid, a result which is consistent with structure IV.

Sauvage, Berger and Boekelheide[3] have also prepared apo-β-erythroidine and isoapo-β-erythroidine, and have converted desmethoxy-β-erythroidine into apo-β-erythroidine. The slight differences of melting points for the apo- and isoapo-compounds are possibly due to the ease of rearrangement of the apo- to the isoapo-compound and to the difficulty of separating them. We have found that apo-β-erythroidine tends to isomerize in hot methanol.

If structure I for β-erythroidine and the corresponding structures for its acid degradation products are not entirely satisfactory, they are at least provocative of new and obvious experiments which would either confirm these structures or lead to final modifications.

Experimental

Apo-β-erythroidine.—A 750-mg. sample of β-erythroidine hydrochloride was dissolved in 5 ml. of concd. hydrobromic acid, and the solution was heated for one hour on the steam-bath. The solution was then diluted with 50 ml. of water, and extracted with six portions of chloroform. The chloroform extract was washed with water and distilled. The crystalline residue weighed 300 mg. and melted at 130–132°. After five recrystallizations from methanol-ethyl ether, apo-β-erythroidine which melted at 144° was obtained; $[\alpha]^{25}D$ +26.6 (*c*, 0.3, CH_3OH).

Anal. Calcd. for $C_{15}H_{15}NO_2$: C, 74.65; H, 6.26; N, 5.81; C-CH_3, 6.2. Found: C, 74.42; H, 6.09; N, 5.70; C-CH_3, 1.00.

Apo- and Isoapo-β-erythroidine.—Four grams of β-erythroidine hydrochloride was dissolved in 24 ml. of concd. hydrobromic acid and the solution was heated in a sealed tube at 100–110° for three hours. After cooling, the solution was diluted with 120 ml. of water and extracted twenty-five times with chloroform. The chloroform extract yielded 1.57 g. of crystalline residue, which was recrystallized from ethyl alcohol. The first crop of crystals weighed 914 mg. and melted at about 126°, and contained apo-β-erythroidine in substantial amount. The mother liquor from the first crop was concentrated to about one-half volume and refrigerated. The yield of crude isoapo-β-erythroidine was 138 mg., m.p. 140–141°. After three recrystallizations from methanol-ethyl ether, the isoapo-β-erythroidine melted constantly at 154–155°; $[\alpha]^{25}D$ +7.1 (*c*, 1.4, CH_3OH).

Anal. Calcd. for $C_{15}H_{15}NO_2$: C, 74.65; H, 6.26; N, 5.80. Found: C, 74.49; H, 6.09; N, 5.72; C-CH_3, 1.65.

Octahydro-apo-β-erythroidine.—A 200-mg. sample of apo-β-erythroidine was dissolved in 15 ml. of water and the solution was acidified with 0.5 ml. of concd. hydrochloric acid. A suspension of 25 mg. of Adams platinum catalyst in 45 ml. of water was added, and hydrogenation at atmospheric pressure resulted in the absorption of four moles of hydrogen. The solution was filtered, made alkaline with sodium bicarbonate, and extracted with chloroform. The crystalline residue, which was left after solvent removal, weighed 118 mg. and was recrystallized twice from ethanol; m.p. 134–136°. The octahydro-apo-β-erythroidine did not give a color reaction with ferric chloride or Ehrlich reagent.

Anal. Calcd. for $C_{15}H_{23}NO_2$: C, 72.26; H, 9.29; N, 5.61; C-CH_3, 6.01. Found: C, 72.28; H, 9.34; N, 5.21; C-CH_3, 2.75.

Octahydro-apo-β-erythroidine Hydrochloride.—The octahydro-apo-β-erythroidine was dissolved in ethanol and a slight excess of hydrogen chloride was added. The hydrochloride crystallized after the solution was refrigerated overnight; m.p. 250–252.5°.

Anal. Calcd. for $C_{15}H_{23}NO_2 \cdot HCl$: C, 63.03; H, 8.46. Found: C, 63.03, 63.27; H, 8.23, 8.52.

Octahydro-apo-β-erythroidine from Hydrogenation of Isoapo-β-erythroidine.—Twenty ml. of water and 5 ml. of concd. hydrochloric acid were mixed and 1 g. of isoapo-β-erythroidine, m.p. 154–155°, was added. Hydrogenation was carried out with 100 mg. of Adams platinum catalyst at 40 lb. pressure. The filtrate from the catalyst was made alkaline with sodium bicarbonate and extracted with ten 25-ml. portions of chloroform. Evaporation of the chloroform yielded 949 mg. of a colorless gum which solidified. This residue was crystallized twice from methanol-petroleum ether to give octahydro-apo-β-erythroidine, m.p. 134–136°. A sample of this hydrogenation product was mixed with a sample of octahydro-apo-β-erythroidine which melted at 134–136° and was obtained from the hydrogenation of apo-β-erythroidine. There was no depression of the melting point of the mixture.

Anal. Calcd. for $C_{15}H_{23}NO_2$: C, 72.26; H, 9.29; N, 5.61. Found: C, 72.36; H, 9.11; N, 5.64.

Oxidation of Apo-β-erythroidine to Formic Acid.—A solution containing 66 mg. of apo-β-erythroidine in 4 ml. of 2 *N* H_2SO_4 was cooled to 0° in an ice-bath. One drop of 2% potassium permanganate solution was added, but the red color did not disappear in five minutes. The solution was allowed to warm to room temperature and oxidation took place slowly. Altogether, 5 ml. of 2% potassium permanganate solution was added. A few drops of 30% hydrogen peroxide solution was added to dispel the last of the permanganate, and the mixture was centrifuged. The supernatant was filtered, combined with the washings and distilled to a residual volume of 5 ml. The concentrate was diluted with 10 ml. of water and distilled again to a 5-ml. volume; this dilution and distillation process was repeated for the third time. The distillates were combined and neutralized to *p*H 6.5 by the addition of 10% sodium hydroxide solution. After adding 10 ml. of methanol, 49 mg. of *p*-bromophenacyl bromide was added and the solution was refluxed for three hours. The solution was evaporated to dryness *in vacuo*, and the residue was triturated with 2 ml. of warm methanol. The sodium bromide was collected on a filter and the filtrate was diluted with petroleum ether. After cooling in an ice-bath, crystals separated which weighed 36 mg. and melted at 130–133°. Recrystallization of this material from methanol gave the *p*-bromophenacyl ester of formic acid, m.p. 134–135°. A mixture of this sample with an authentic specimen showed no depression of the melting point.

Acknowledgment.—We are indebted to Messrs. D. F. Hayman, W. Reiss and H. C. Clark for the microanalyses.

Summary

β-Erythroidine has been degraded to apo-β-erythroidine and isoapo-β-erythroidine by reaction

(3) Sauvage, Berger and Boekelheide, *Science*, **109**, 627 (1949).

with hydrobromic acid. The hydrogenation of apo- and isoapo-β-erythroidine yielded the same compound, octahydro-apo-β-erythroidine. Oxidation of apo-β-erythroidine gave formic acid.

Interpretation of these reactions and degradation products of β-erythroidine allows one to formulate tentative structures for these products.

RAHWAY, N. J. RECEIVED JULY 18, 1950

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

Vitamin B_{12}. XIII. Additional Data on Vitamin B_{12a}

BY EDWARD A. KACZKA, ROBERT G. DENKEWALTER, ARNOLD HOLLAND AND KARL FOLKERS

In a recent communication,[1] crystalline vitamin B_{12a} was described as a reaction product of vitamin B_{12} and hydrogen over a platinum catalyst. The described physical properties of vitamin B_{12a} showed that it could be differentiated from vitamin B_{12} although these two compounds are obviously very closely related in composition and properties. The results of the first preliminary biological tests which were reported,[1] were not intended as a critical and final evaluation, but to show that vitamin B_{12a} possesses high vitamin B_{12} activity.

Vitamin B_{12a} has been examined further, and samples, including the one described,[1] have been found to be about 98% pure by solubility analyses. Vitamin B_{12a} separates from aqueous acetone in needle-like or bladed crystals which belong to the orthorhombic systems. The color of the crystals is somewhat darker red than those of vitamin B_{12}. On the micro-block, the crystals start to darken at about 200°, with more decomposition appearing to take place than with vitamin B_{12}, but do not melt below 300°. The refractive indices of two samples (dried *in vacuo* at room temperature) which resulted from two separate hydrogenation experiments are given in Table I, When the crystals of sample 2 were heated for two hours at 100° *in vacuo*, the refractive indices were found to be: α, 1.604 ± 0.004; β, 1.640 ± 0.004; γ, 1.654 ± 0.004. This heating of crystals of vitamin B_{12a} does not seem to alter very much the β and γ indices, but does seem to cause a progressive increase in the α index. The refractive indices may be used as a criterion for identity and reproducibility of vitamin B_{12a}.

TABLE I

REFRACTIVE INDICES OF VITAMIN B_{12a}

Sample	α	β	γ
1	1.580 ± 0.002	1.640 ± 0.002	1.657 ± 0.002
2	1.580 ± 0.002	1.640 ± 0.002	1.656 ± 0.002
Vitamin B_{12a} (from *S. griseus*)	1.584 ± 0.002	1.640 ± 0.002	1.657 ± 0.002

Amorphous and crystalline concentrates from culture broths of *Streptomyces griseus*, which showed vitamin B_{12} activity in microbiological assays, yielded a red crystalline compound after further fractional crystallization. The absorption spectrum of this red crystalline product is like that of vitamin B_{12a}, but differs from that of vitamin B_{12} as shown in Fig. 1. The slight variations in the absorption spectra of vitamin B_{12a} and the crystals from *S. griseus* in the regions 265–275 mμ, 300–320 mμ and 350–358 mμ are influenced by

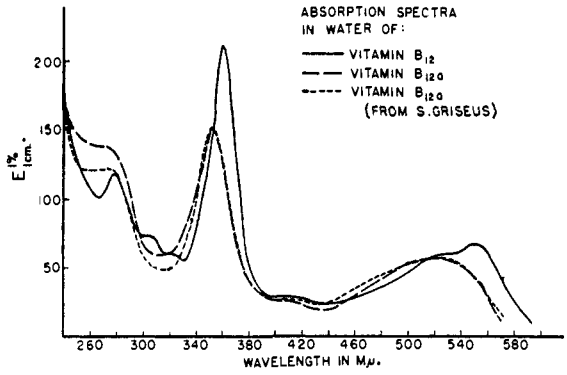


Fig. 1.

slight variations in the *p*H of the solutions and this influence is demonstrated in Fig. 2. During the early work, the *p*H variations of water solutions of samples of vitamin B_{12a} were not determined, It is evident that vitamin B_{12a} and the crystals from *S. griseus* cannot be differentiated by absorption spectrum (Fig. 1) or refractive indices (Table I).

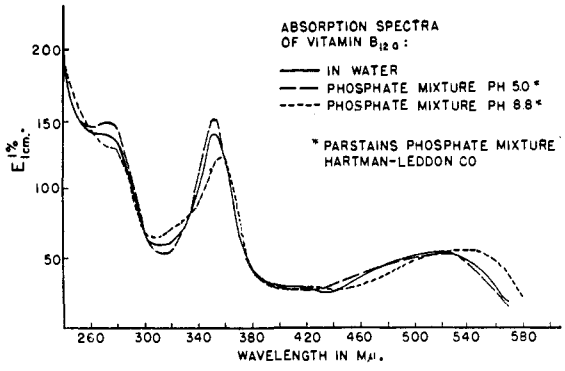


Fig. 2.

A sample (NP-92-58-4)[2] of vitamin B_{12b},[3] has been compared with vitamin B_{12a} obtained from the hydrogenation reaction with the following results: The two samples cannot be differentiated by absorption spectrum as illustrated by Fig. 3. The sample of vitamin B_{12a} used for this comparison was found to be 98.5% pure. When powdered samples of vitamin B_{12a} were dried at 100° for two hours *in vacuo* and then dissolved in water, the spectrum was found to be, on immediate

(1) Kaczka, Wolf and Folkers, THIS JOURNAL, 71, 1514 (1949).

(2) Through the courtesy of Dr. T. H. Jukes of the Lederle Laboratories, Division of the American Cyanamid Company.

(3) Pierce, Page, Stokstad and Jukes, THIS JOURNAL, 71, 2953 (1949).