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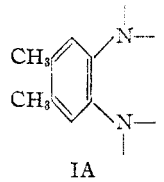
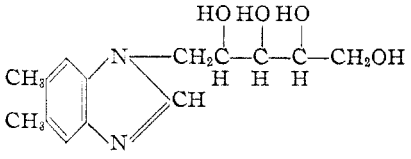
Carcinolytic Compounds. II. 1-(1'-Glycityl)-benzimidazoles

BY FREDERICK W. HOLLY, ELIZABETH W. PEEL, JOSEPH J. CAHILL AND KARL FOLKERS

The effectiveness of 6,7-dichloro-9-(1'-D-sorbityl)-isoalloxazine, a riboflavin analog, in causing regression of lymphosarcoma in mice has been reported.[1] Since the 1,2-diamino-4,5-dimethylbenzene moiety (IA) is a component of riboflavin and of vitamin B_{12},[2] and 5,6-dimethylbenzimidazole has been obtained by degradation of vitamin B_{12},[2] it was considered of interest to synthesize some 1-glycitylbenzimidazoles for testing biologically as inhibitors in both riboflavin and vitamin B_{12} systems. Accordingly, 1-(1'-D-ribityl)-5,6-dimethylbenzimidazole (I), 1-(1'-D-sorbityl)-5,6-dichlorobenzimidazole (II), 1-(1'-D-arabityl)-5,6-dichlorobenzimidazole (III) and 1-(1'-D-xylityl)-5,6-dichlorobenzimidazole (IV) were synthesized.



IA



I

II, R = 1'-D-sorbityl
III, R = 1'-D-arabityl
IV, R = 1'-D-xylityl

For testing these compounds against lymphosarcoma in mice, we are indebted to Dr. Gladys A. Emerson of the Merck Institute for Therapeutic Research. These four compounds were ineffective in enhancing regression of established lymphosarcoma (6C3H-ED) implants in mice of the C3H strain which were fed a diet deficient in riboflavin.

1-(1'-D-Ribityl)-5,6-dimethylbenzimidazole (I) was prepared by a reaction of 2-amino-4,5-dimethyl-N-(1'-D-ribityl)-aniline with formic acid in aqueous hydrochloric acid.[3]

The three dichloro analogs II, III and IV were also synthesized by treatment of the corresponding diamine with formic acid in aqueous hydrochloric acid. The diamines were obtained by hydrogenation of the nitroanilines: 2-nitro-4,5-dichloro-N-(1'-D-sorbityl)-aniline,[1] 2-nitro-4,5-dichloro-N-(1'-D-arabityl)-aniline and 2-nitro-4,5-dichloro-N-(1'-D-xylityl)-aniline. The D-arabityl- and D-xylityl-nitroanilines were synthesized by reaction of 1,2-dinitro-4,5-dichlorobenzene with D-arabinamine and D-xylamine, respectively. Hydrogenation of D-arabinose and D-xylose in liquid ammonia over a nickel catalyst, as described[1] for other aldoses, was a satisfactory means of producing D-arabinamine and D-xylamine.

Experimental

Preparation of Glycamines.—D-Arabinamine and D-xylamine were prepared by hydrogenation of D-arabinose and D-xylose, respectively, in liquid ammonia,[1] and were used as oils without purification.

2-Nitro-4,5-dichloro-N-(1'-D-arabityl)-aniline.—D-Arabinamine, obtained from 50 g. of D-arabinose as described above, was dissolved in 200 ml. of 50% ethanol and the solution was added to a solution of 30 g. of 1,2-dinitro-4,5-dichlorobenzene in 300 ml. of methanol. The resulting solution was heated on a steam-bath for one hour, during which time a crystalline precipitate formed. After the mixture had cooled to 25°, the crystalline product was collected on a filter and washed with ethanol and with ether to give 32 g. of 2-nitro-4,5-dichloro-N-(1'-D-arabityl)-aniline, m.p. 226–236° (micro block). This product was used to prepare the corresponding benzimidazole. A sample recrystallized twice from methanol melted at 236–238°. *Anal.* Calcd. for $C_{11}H_{14}N_2O_6Cl_2$: C, 38.73; H, 4.14; N, 8.21. Found: C, 39.29; H, 4.32; N, 8.73. This sample was crystallized again from methanol to give a product melting at 237–238°. *Anal.* Found: C, 37.91, 37.99; H, 3.91, 3.95. A sample of the crude product of m.p. 226–236° recrystallized four times from 50% aqueous acetic acid and once from glacial acetic acid melted at 234–237°. *Anal.* Found: C, 39.28; H, 3.86; N, 7.98. The difficulty in obtaining reproducible analytical data has not been resolved.

2-Nitro-4,5-dichloro-N-(1'-D-xylityl)-aniline.—The procedure described above for preparation of the D-arabityl isomer was used to prepare 2-nitro-4,5-dichloro-N-(1'-D-xylityl)-aniline, m.p. 164–166° (micro block). *Anal.* Calcd. for $C_{11}H_{14}N_2O_6Cl_2$: C, 38.73; H, 4.14; N, 8.21. Found: C, 38.94; H, 4.08; N, 8.46.

1-(1'-Glycityl)-benzimidazoles.—A preparation of 1-(1'-D-sorbityl)-5,6-dichlorobenzimidazole is described, and is representative of the procedures used. A solution of 20 g. of 2-nitro-4,5-dichloro-N-(1'-D-sorbityl)-aniline was hydrogenated at 40 p.s.i. in 200 ml. of methanol over 3 g. of palladium–Darco (5%) catalyst. The catalyst was removed by filtration, and the filtrate was concentrated *in vacuo* to a

TABLE I

1-(1'-GLYCITYL)-BENZIMIDAZOLES

Benzimidazole	Formula	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found	M. p.[a]
1-(1'-D-Ribityl)-5,6-dimethyl-[b]	$C_{14}H_{20}N_2O_4$	59.98	60.10	7.19	7.10	10.00	10.33	210–212°[c]
1-(1'-D-Sorbityl)-5,6-dichloro-	$C_{13}H_{16}N_2O_6Cl_2 \cdot {}^1/_2H_2O$	43.35	43.47	4.76	5.17	7.78	7.91	206–207°[d]
1-(1'-D-Arabityl)-5,6-dichloro-	$C_{12}H_{14}N_2O_4Cl_2$	44.88	45.00	4.39	4.17	8.73	8.85	204–206°[e]
1-(1'-D-Xylityl)-5,6-dichloro-	$C_{12}H_{14}N_2O_4Cl_2$	44.88	45.14	4.39	4.31	8.73	8.85	245–250°[f]

[a] Melting points were determined on a micro block. [b] The intermediate diamine was prepared by hydrogenation of 4,5-dimethyl-2-phenylazo-N-(D-ribityl)-aniline (Karrer and Meerwein, *Helv. Chim. Acta*, 19, 266 (1936)). [c] Recrystallized from ethanol–water. [d] Recrystallized from acetic acid–water; transition beginning at 145°. [e] Recrystallized from ethanol–water. [f] Recrystallized from acetic acid–ether.

(1) Holly, Peel, Mozingo and Folkers, THIS JOURNAL, **72**, 5416 (1950).

(2) Brink and Folkers, *ibid.*, **71**, 2951 (1949).

(3) Phillips, *J. Chem. Soc.*, 2393 (1928).

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

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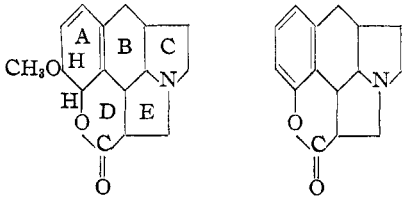
Erythrina Alkaloids. XX. Apo- and Isoapo-β-erythroidine

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.

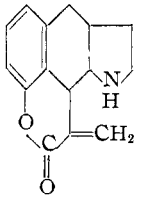


I

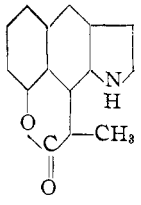
II

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).