40. *Synthetic Experiments in the B Group of Vitamins. Part I. Riboflavin.*

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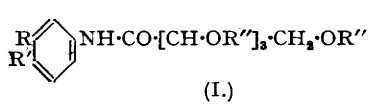
A synthesis of *N*-(polyhydroxyalkyl)arylamines is described. Acetylation of an arylamide of an aldonic acid, followed by treatment with phosphorus pentachloride, gives the corresponding imidochloride. Catalytic hydrogenation to an acetylated secondary amine, followed by deacetylation, gives the required *N*-(polyhydroxyalkyl)arylamine.

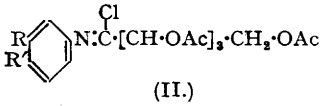
N-(*d*-Ribityl)-*o*-4-xylidine, so prepared, is converted into 6-(*N*-*d*-ribitylamino)-3 : 4-dimethylazobenzene by known methods, and the latter compound undergoes a reductive condensation with alloxantin, yielding riboflavin after complete oxidation of any leuco-riboflavin.

A synthesis of *N*-(polyhydroxyalkyl)arylamines (III, R″ = H) suitable for the preparation of *iso*alloxazines, including riboflavin, was first described by us in the patent literature [Brit. Pat. 550,169 (6485/41); printed January, 1943], and consisted in converting acetylated arylamides of aldonic acids (cf. I, R″ = Ac) into imidochlorides (II), which were reduced (III, R″ = Ac) and deacetylated to (III, R″ = H). Recently, however, a similar method of preparing *N*-(*d*-ribityl)-*o*-4-xylidine, the intermediate required for the synthesis of riboflavin, was published by Tishler, Wendler, Ladenburg, and Willman (*J. Amer. Chem. Soc.*, 1944, **66**, 1328).

In view of their statement regarding lack of details of our procedure, it is desirable to record a number of experiments on the synthesis of compounds of type (III), R″ = H.



(I.)



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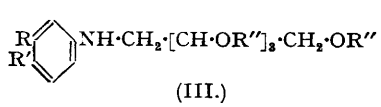

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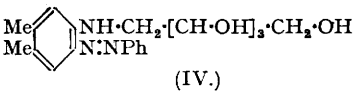

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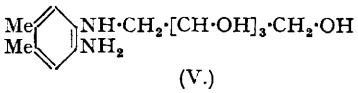

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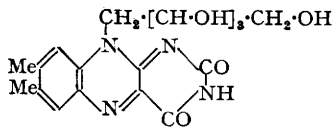

(VI.)

The conversion of *N*-(*d*-ribityl)-*o*-4-xylidine into riboflavin has long since been reported by Karrer and Meerwein (*Helv. Chim. Acta*, 1935, **18**, 1130; 1936, **19**, 264). They coupled the secondary base with a benzenediazonium salt to form the azo-compound (IV), reduced the latter to the diamine (V), which condensed with alloxan, giving riboflavin (VI). We wish to report a new observation regarding these stages.* The azo-compound (IV) reacted with reduced forms of alloxan, such as alloxantin, in the presence of palladised charcoal with the occurrence of a reductive condensation of (IV), even in the absence of hydrogen. When the reaction was carried out in an atmosphere of nitrogen with an excess of alloxantin, the diamine (V) and alloxan reacted *in situ*, and riboflavin was directly obtained after the nitrogen had been replaced by air to oxidise any leuco-riboflavin.

Returning to the general synthesis of *N*-polyhydroxyalkylarylamines, these experiments were undertaken in order to develop a synthesis of riboflavin in which the *d*-ribityl side chain is introduced into the *iso*alloxazine molecule without the use of the difficulty accessible *d*-ribose. Alternative solutions of this problem have already been reported (see, *e.g.*, Weygand, *Ber.*, 1940, **73**, 1259; B.P. 545,360, 551,491; U.S.P. 2,261,608; Tishler *et al.*, *loc. cit.*) and the present experiments exploit the use of the readily available *d*-arabonolactone.

By adopting the general procedure outlined in the opening paragraph of this paper, the following compounds have been prepared: N-d-*arabityl-aniline*, -p-*toluidine*, -o-4-*xylidine*, and -p-*anisidine*, N-d-*ribityl*-p-*toluidine*, and *N*-*d*-ribityl-*o*-4-xylidine. The characteristics of the last compound were in agreement with those recorded in the literature (Karrer and Meerwein, Tishler *et al.*, *locc. cit.*). When *o*-4-xylidine and *d*-ribonolactone were allowed to react at a higher temperature (above 120°) than was usually employed in the preparation of the substituted anilide, epimerisation was observed and an appreciable amount of *d*-arabono-*o*-4-xylidide was isolated, identical with an authentic specimen prepared from *d*-arabonolactone.

Since the completion of these experiments Work has described (J., 1942, 429) the synthesis of secondary bases by the reduction of imidochlorides derived from certain substituted quinoline-4-carboxyamides, with stannous chloride and ethereal hydrogen chloride. The constitutional factors which determine whether the stannous chloride reduction product is an aldehyde (Sonn and Müller reaction) or a base appear to be uncertain, but it seems fairly clear that the method of catalytic hydrogenation of imidochlorides has wider applicability in the synthesis of secondary amines.

Experimental.

d-*Arabonanilide*.—Following the general method of van Marle (*Rec. Trav. chim.*, 1920, **39**, 549), an intimate mixture of *d*-arabonolactone (1 mol.) and aniline (1 mol.) was heated at 100° for 6 hours. The solid product was powdered, washed with benzene, and crystallised from alcohol, from which d-*arabonanilide* separated in excellent yield, in colourless needles, m. p. 198° (Found: C, 54·9; H, 6·4. $C_{11}H_{15}O_5N$ requires C, 54·8; H, 6·2%).

Tetra-acetyl-d-*arabonanilide*.—*d*-Arabonanilide (2·5 g.) was added gradually to an ice-cooled solution of anhydrous zinc chloride (1·0 g.) in acetic anhydride (15 c.c.). After cooling for 1 hour, the mixture was kept at room temperature for 48 hours and poured into water. The precipitated *tetra-acetyl*-d-*arabonanilide* (3·8 g., m. p. 160°) crystallised from alcohol in colourless needles, m. p. 161° (Found: C, 56·0; H, 5·8. $C_{19}H_{23}O_9N$ requires C, 55·6; H, 5·6%).

N-*Phenyltetra-acetyl*-d-*arabonylimidochloride*.—A mixture of finely powdered tetra-acetyl-*d*-arabonanilide (4·09 g.), phosphorus pentachloride (2·08 g.; *ca.* 1 mol.), and toluene (50 c.c.) was gently heated on the water-bath for 1 hour; the solid gradually dissolved with evolution of hydrogen chloride. Toluene and phosphorus oxychloride were removed under reduced pressure, leaving a yellow oil which solidified on cooling. An ethereal solution of this was filtered clear and treated with light petroleum (b. p. 40—60°); the *imidochloride* then separated in colourless needles (2·4 g.), m. p. 102° (Found: Cl, 8·2. $C_{19}H_{22}O_8NCl$ requires Cl, 8·3%).

N-(*Tetra-acetyl*-d-*arabityl*)*aniline*.—A solution of the above imidochloride (1·5 g.) in ethyl acetate (40 c.c.) was shaken with anhydrous sodium acetate (0·3 g.) and palladised charcoal (25%; 0·2 g.) in a hydrogen atmosphere until no more hydrogen was absorbed. Filtration and evaporation of the solution yielded a yellow oil which solidified on cooling and rubbing. This crystallised from methanol in needles, m. p. 75—76° (Found: C, 57·6; H, 6·1; N, 4·0. $C_{19}H_{25}O_8N$ requires C, 57·7; H, 6·3; N, 3·5%).

N-(d-*Arabityl*)*aniline*.—*N*-(Tetra-acetyl-*d*-arabityl)aniline (1·7 g.) was heated under reflux for ¾ hour with a solution of crystalline barium hydroxide (6·0 g.) in water (100 c.c.). Carbon dioxide was passed into the hot solution, barium carbonate removed, the filtrate evaporated to dryness under reduced pressure, and the residue triturated with hot alcohol. N-(d-*Arabityl*)*aniline* (0·5 g.) crystallised in needles, m. p. 157—159°, from the filtered alcoholic solution on cooling (Found: C, 58·2; H, 7·6; N, 6·0. $C_{11}H_{17}O_4N$ requires C, 58·2; H, 7·5; N, 6·2%).

The compounds described below were prepared in a similar manner to that of the analogous compound described above. A better procedure for the preparation of the imidochlorides consisted in heating the toluene suspension of the

* Cf. B.P. 550,836.

corresponding substituted amide with slightly more than 1 mol. of phosphorus pentachloride at 30—50° for ½—1 hour. Toluene and phosphorus oxychloride were removed at 1—2 mm. pressure. The addition of toluene and evaporation under reduced pressure were repeated twice to remove all phosphorus oxychloride and the crude residual imidochloride was usually hydrogenated to the secondary amine without further treatment. (Failure to remove all the phosphorus oxychloride appears seriously to diminish the speed of hydrogenation.) d-*Arabono*-p-*toluidide*, crystallised from alcohol, m. p. 199—200° (Found: N, 5·7. $C_{12}H_{17}O_5N$ requires N, 5·5%); *tetra-acetyl*-d-*arabono*-p-*toluidide*, needles from alcohol, m. p. 167—168° (Found: N, 3·7. $C_{20}H_{25}O_7N$ requires N, 3·3%); N-(p-*tolyl*)*tetra-acetyl*-d-*arabonylimidochloride*, colourless needles, m. p. 82°, from ether–light petroleum (Found: Cl, 8·1. $C_{20}H_{24}O_6NCl$ requires Cl, 8·0%); N-(*tetra-acetyl*-d-*arabityl*)-p-*toluidine*, needles from methanol, m. p. 72° (Found: C, 59·0; H, 6·4; N, 3·6. $C_{20}H_{27}O_8N$ requires C, 58·7; H, 6·6; N, 3·4%); N-(d-*arabityl*)-p-*toluidine*, crystallised from alcohol, m. p. 179° (Found: C, 60·0; H, 7·7; N, 5·9. $C_{13}H_{19}O_4N$ requires C, 59·8; H, 7·9; N, 5·8%).

d-*Ribono*-p-*toluidide*, needles from alcohol, m. p. 157—158° (Found: C, 56·9; H, 6·9. $C_{13}H_{17}O_5N$ requires C, 56·5; H, 6·7%); *tetra-acetyl*-d-*ribono*-p-*toluidide*, needles from alcohol, m. p. 120—121° (Found: N, 3·6. $C_{20}H_{25}O_9N$ requires N, 3·4%); the imidochloride was hydrogenated without complete purification to the acetylated secondary base, which was hydrolysed to N-(d-*ribityl*)-p-*toluidine*, m. p. 138—140° after crystallisation from alcohol (Found: C, 59·8; H, 7·9; N, 5·95. $C_{12}H_{19}O_4N$ requires C, 59·8; H, 7·8; N, 5·8%). *d*-Ribono-*o*-4-xylidide, needles from alcohol, m. p. 160° (Found: N, 5·2. Calc. for $C_{13}H_{19}O_5N$: N, 5·2%); tetra-acetyl-*d*-ribono-*o*-4-xylidide, needles from alcohol, m. p. 112° (Found: N, 3·5. Calc. for $C_{21}H_{27}O_9N$: N, 3·2%); the corresponding imidochloride and acetylated secondary base (m. p. 99°) were obtained by the methods already described, and the latter hydrolysed by barium hydroxide solution or alcoholic sodium hydroxide to *N*-(*d*-ribityl)-*o*-4-xylidine, m. p. 143°, after crystallising from water (56% yield, calculated on *d*-ribonolactone).

d-*Arabono*-o-4-*xylidide*, needles from alcohol or water, m. p. 206° (Found: N, 5·3. $C_{13}H_{19}O_5N$ requires N, 5·2%); *tetra-acetyl*-d-*arabono*-o-4-*xylidide*, prismatic needles from alcohol, m. p. 151° (Found: C, 57·6; H, 6·2; N, 3·4. $C_{21}H_{27}O_9N$ requires C, 57·65; H, 6·2; N, 3·2%); N-(o-4-*xylyl*)*tetra-acetyl*-d-*arabonylimidochloride*, m. p. 83—84° after one crystallisation from ethyl acetate–light petroleum (Found: Cl, 7·4. $C_{21}H_{26}O_8NCl$ requires Cl, 7·8%); N-(*tetra-acetyl*-d-*arabityl*)-o-4-*xylidine*, needles, m. p. 91°, from 80% methanol (Found: C, 59·3; H, 6·9; N, 3·55. $C_{21}H_{29}O_8N$ requires C, 59·7; H, 6·9; N, 3·3%); N-(d-*arabityl*)-o-4-*xylidine*, prisms, m. p. 140—141° (Found: C, 61·0; H, 8·2; N, 5·3. $C_{13}H_{21}O_4N$ requires C, 61·2; H, 8·2; N, 5·5%).

d-*Arabono*-p-*anisidide*, needles from alcohol, m. p. 185—187° (Found: C, 53·1; H, 6·2. $C_{13}H_{17}O_6N$ requires C, 53·0; H, 6·2%); *tetra-acetyl*-d-*arabono*-p-*anisidide*, from alcohol, m. p. 180° (Found: C, 54·5; H, 5·8; N, 3·4. $C_{20}H_{25}O_{10}N$ requires C, 54·8; H, 5·7; N, 3·2%); this compound gave, in the usual manner *via* the imidochloride and subsequent hydrolysis with alcoholic sodium hydroxide, an overall yield of 60% of N-(d-*arabityl*)-p-*anisidine*, m. p. 150° (Found: C, 56·0; H, 7·3; N, 5·4. $C_{12}H_{19}O_5N$ requires C, 56·2; H, 7·4; N, 5·4%).

Epimerisation in the Preparation of d-*Ribono*-o-4-*xylidide*.—A mixture of equimolecular amounts of *d*-ribonolactone and *o*-4-xylidine was heated at 130° for 5 hours, and the product extracted with boiling butanol and filtered. *d*-Ribono-*o*-4-xylidide, m. p. 158—160° (cf. above), crystallised from the filtrate. The butanol-insoluble fraction was crystallised from alcohol, yielding (about 35%) *d*-arabono-*o*-4-xylidide, m. p. 205°, which was not depressed in admixture with an authentic specimen prepared from *d*-arabonolactone.

Synthesis of Riboflavin from Alloxantin and 6-(N-d-*Ribitylamino*)-3 : 4-*dimethylazobenzene*.—The azo-compound was prepared by coupling diazotised aniline with *N*-(*d*-ribityl)-*o*-4-xylidine (cf. Karrer, *Helv. Chim. Acta*, 1935, **18**, 1130). A mixture of it (0·5 g.) and alloxantin (2·0 g.) with palladised charcoal (0·2 g.; 10%) in N-alcoholic hydrogen chloride (5 c.c.) and alcohol (80 c.c.) was shaken in an atmosphere of nitrogen at 60—70° for 5 hours. The nitrogen was then displaced by air, and agitation continued for 4 hours at room temperature. The catalyst, mixed with crude riboflavin, was filtered off, and extracted with a mixture of water (15 c.c.) and N-sodium hydroxide (1 c.c.). The filtered alkaline solution was acidified with acetic acid and riboflavin was soon precipitated as an orange-yellow solid (0·15 g.), m. p. 280°. Crystallisation from aqueous acetic acid raised the m. p. to 282° (corr.) (Found: C, 54·0; H, 5·45; N, 15·0. Calc. for $C_{17}H_{20}O_6N_4$: C, 54·4; H, 5·3; N, 14·9%).

We are grateful to Mr. E. G. Hughes for information concerning the *p*-anisidine derivatives, and to Mr. J. D. H. Kirkland for assistance in the experimental work.

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