An Improved Synthesis of 8-Amino-8-demethylriboflavin

Sabu KASAI,* Bernhard J. FRITZ, and Kunio MATSUI
Research Institute for Atomic Energy, Osaka City University, Sugimoto, Sumiyoshi-ku, Osaka 558
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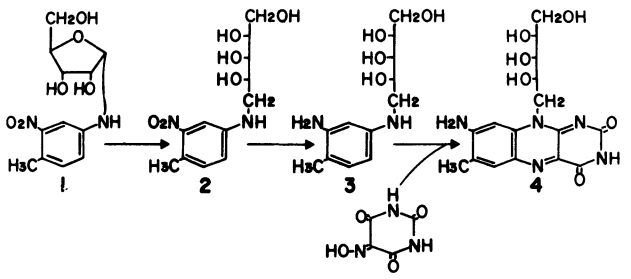
Synopsis. 8-Amino-8-demethylriboflavin was synthesized from 3-nitro-4-methylaniline *N*-D-riboside by selective reduction of the *N*-glycoside with sodium borohydride, followed by hydrogenation of the nitro group in the presence of Raney's catalyst. The obtained phenylenediamine was condensed with violuric acid to give the flavin in a high yield.

8-Amino-8-demethylriboflavin (**4**) has been used as a riboflavin (RF) analogue to study reaction mechanisms of flavoenzymes,[2] flavin-protein interactions,[3,4] or antimicrobial action.[1,5] **4** has a low redox potential (about 100 mV lower than RF),[1] and both the conjugation system and the stability of the semiquinone differ from those of RF.[1,2,12] Moreover, **4** is a key compound from which many 8-substituted flavins can be derived via diazotization,[9,10] and 8-substituted flavins such as the naturally occuring 8-hydroxy-8-demethylriboflavin,[6-8] have been used widely. It is noteworthy that most flavins used in biochemical studies carry a ribityl group at 10-position of the isoalloxazine ring, whereas in purely chemical research usually lumiflavin derivatives are used.

4 was already synthesized by Berezovskii et al.,[11] but, using their method, we achieved only low yields of 5 to 10%. In the final step, a dark powder was obtained, making the purification tedious. If the reduction of the riboside is carried out in one step as given by Berezovskii et al., there exists the possibility that the nitro group is reduced first, followed by migration of the ribosyl group. Upon condensation with violuric acid, a product of the lumichrome type would be obtained. We figured this to be the main reason for the low yield of above method, and aimed to reduce first the riboside selectively, thus preventing the migration of the ribosyl group. This was achieved by using excess sodium borohydride in refluxing ethanol. The reaction can also be carried out with sodium cyanotrihydroborate, where smaller amounts of the reductant are sufficient. Samples prepared according to our



method gave analytical results and absorption spectra identical to samples prepared by us according to Berezovskii et al.

Experimental

Mass spectrum (field desorption) was taken on a JEOL JMS-01SG-2; IR spectrum was recorded on a JASCO model IRA grating infrared spectrophotometer; UV spectra were recorded on a Hitachi 320 recording spectrophotometer; 400 MHz ^{1}H NMR spectra were taken with a JEOL model JMM GX 400. Melting points were measured with a Yanaco MP micro melting point apparatus.

3-Nitro-4-methyl-*N*-ribitylaniline (2). Two grams of 3-nitro-4-methylaniline *N*-D-riboside (**1**)[11] was dissolved in 20 ml ethanol and sodium borohydride (1.5 g) was added. The reaction mixture was refluxed for 1.5 h under stirring, during which the color of the solution changed from orange to red. After cooling to room temperature, the solution was neutralized with 1 M (mol dm^{-3}) hydrochloric acid and evaporated to dryness. A small amount of methanol was added to the residue. Salt precipitated, which was filtered and washed. Filtrates were combined, evaporated, redissolved in a 8:2 mixture of dichloromethane and methanol, charged on a silica-gel column (Wakogel C-200, Wako Pure Chemical Industries, Ltd., 3 cm×20 cm), and developed with the same solvent. Red fractions were collected and evaporated. Attempts to crystallize the syrup from several solvents were unsuccessful. FD-MS: Found *m/z* 286.0. Calcd for $C_{12}H_{18}O_6N_2$: M, 286.28.; IR (Nujol) 1545 and 1350 cm^{-1} (NO_2).

The syrup obtained above was dissolved in 10 ml ethanol and hydrogen chloride gas was introduced into the solution under cooling, upon which the color of the solution changed from red to light brown. 30 ml of diisopropyl ether were added to the solution and 3-nitro-4-methyl-*N*-ribitylaniline hydrochloride (**2'**) separated as syrup, which was crystalized by adding seed crystals. The crystals were filtered off, washed with diisopropyl ether and dried over calcium chloride in vacuo. Yield 2.0 g (88%): mp 138—140 °C; ^{1}H NMR (D_2O) δ=2.61 (3H, s, CH_3), 3.67 (2H, dd, J=12 and 4 Hz, 5'CH_2), 3.79 (4H, m, 1'CH_a, 2'-4'CH), 4.14 (1H, m, 1'CH_b), 7.65 (1H, d, J=8 Hz, 5CH), 7.74 (1H, dd, J=8 and 2 Hz, 6CH), 8.18 (1H, d, J=2 Hz, 2CH). Found: C, 44.63; H, 5.96; N, 8.67%. Calcd for $C_{12}H_{19}N_2O_6Cl$: C, 44.66; H, 5.93; N, 8.68%.

8-Amino-8-demethylriboflavin (4). One gram of **2'** was suspended in 20 ml methanol and 120 mg powdered NaOH was added. Color of the solution was changed from colorless to red and the solution became turbid with crystals of NaCl, which were filtered off and washed with methanol. The combined filtrates were hydrogenated at 50 °C for 30 min under 50 kg cm^{-2} in the presence of Raney's catalyst, which was prepared from 4.0 g of a 50% alloy with 1 M NaOH. The catalyst was filtered from the colorless solution, washed with methanol, and the combined filtrates were evaporated in vacuo. The diamine obtained was easily oxidized, and therefore was used for the next reaction without isolation. The residue, dissolved in 20 ml methanol, was added to a hot solution of 0.6 g violuric acid in 40 ml of a 1 : 3 (v/v) mixture of methanol and water. Soon after addition, the color of the reaction mixture turned dark, fluorescence developed, and fine orange crystals appeared. After reflux for 4 h the reaction mixture was cooled and allowed to stand at room temperature overnight. Crystals (760 mg) were collected by filtration, washed with methanol and dried over $CaCl_2$. Another 210 mg of the crystals were recovered from the mother liquid. Total yield 970 mg (81%). For elemental analysis, the product was recrystallized from hot water.

^{1}H NMR (DMSO-d_6)(*indicates exchangable H) δ=2.48 (3H, s, CH_3), 3.47 (1H, m, 5'CH_a), 3.58 (1H, m, 5'CH_b), 3.67 (2H, m, 3',4'CH), 4.26 (1H, m, 2'CH), 4.32* (1H, t, J=6 Hz, 5'OH), 4.52 (1H, d, J=14 Hz, 1'CH_a), 4.68 (1H, d, J=4 Hz, 1'CH_b), 4.76* (2H, m, OH), 4.90* (1H, d, J=5 Hz, OH), 6.97 (1H, s, ϕ-H), 6.99* (2H, s, NH_2), 7.63 (1H, s, ϕ-H), 10.73* (1H, s, 3NH) (for assignments, see also[13]). UV (0.2M phosphate buffer pH 7.0) 235nm (ε_{mM} 31.7), 296 (8.9), and 476 (43.9); (6M HCl) 246 (36.5), 308 (18.9), 384 (4.6), and 477 (32.3); (0.1M NaOH) 248 (46.9), 292sh (6.3), and 475 (46.9); mp over 300 °C. Found: C, 50.78; H, 4.96; N, 18.41%. Calcd for $C_{16}H_{19}N_5O_6$: C, 50.92; H, 5.08; N, 18.56%. Literature values[10]; mp over 350 °C; UV (water) 252 (49.0), 296 (8.8), and 490 (42.0).

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