8α-HYDROXYFLAVINMONONUCLEOTIDE AND RELATED COMPOUNDS

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Summary 2',3',4'-Triacetyl-FMN has been transformed by selective radical bromination into 2',3',4'-triacetyl-8α-bromo-FMN, and the following hydrolysis of the latter has afforded 8α-hydroxy-FMN. The presence of the hydroxy group in the 8α position of 8α-hydroxy-FMN is confirmed by its acetylation into 2',3'-diacetyl-8α-acetoxyriboflavin-4',5'-cyclophosphate. The absorption spectra of the synthesized compounds have shown the reduction of the extinction ratios of the first and second absorption maxima in comparison with the extinction of the same maxima for 8α-hydroxyriboflavin. Unlike FMN, fluorescence quenching for 8α-hydroxy-FMN has been found.

The structure of some flavoproteins involves the following derivatives of 8α-amino acids, such as 8α-(N3-L-histidyl)-flavin from succinate dehydrogenase (1, 2), 8α-(N1-L-histidyl)-flavin from β-cyclopiazonate oxidocyclase (3) and thiamine dehydrogenase (4, 5), 8α-(S-L-cysteinyl) flavin from monoamine oxidase (6), 8α-(S-L-cysteinyl) flavin thiohemiacetal and its thiazolidine form (7) from Chromatium flavocytochrome c552. Probably flavins with 8α-functional group are either the metabolites or the participants of the biosynthesis of similar 8α-substituted flavins. The biosynthesis of 8α-(N3-L-histidyl) riboflavin (SD-riboflavin) or its 5'-monophosphate and also SD-FAD may occur as a result of the condensation of histidine with 8α-hydroxyriboflavin or with 8α-hydroxy-FMN and 8α-hydroxy-FAD (8), respectively. By hard acid hydrolysis, SD-flavin and 8α-(S-cysteinyl) flavin are transformed into 8α-hydroxyriboflavin (9); this compound is also formed during the long storage of aqueous solutions of 2',3',4',5'-tetraacetyl-8-formyl (nor) riboflavin at room temperature (10). Earlier some 8α-substituted flavins have been prepared (1, 11, 12). Recently, we synthesized 8α-hydroxyriboflavin by the hydrolysis of 2',3',4',5'-tetraacetyl-8α-bromoriboflavin (13).

For the first time, we have synthesized 8α-hydroxyflavinmononucleotide (8α-hydroxy-FMN) (III) and its derivatives. By selective radical bromination using the method (2), 2',3',4'-triacetyl-FMN (I) has been transformed into 2',

3', 4'-triacetyl-8α-bromo-FMN (II), and the following hydrolysis of the latter has afforded disodium salt of the compound (III).

In comparison with the NMR spectrum of the original flavin (I) in the NMR spectrum of the compound (II) (CD$_3$OD), the disappearance of the three-proton peak of the 8-CH$_3$ group (2.45 ppm) and the appearance of the two-proton peak of the 8-CH$_2$ group (3.65 ppm) has been found. In view of the easy proton lability in the 8-CH$_3$ group and its deuteration (14), it may be concluded that the bromination of 2',3',4'-triacetyl-FMN (I) occurs at the 8-CH$_3$ group as well as the bromination of 2',3',4',5'-tetraacetylriboflavin which has resulted in 2',3',4',5'-tetraacetyl-8α-bromoriboflavin under the same conditions (2).

The substitution of bromine in 8α-bromo-FMN (II) by the hydroxy group took place under heating of the aqueous solution of 2',3',4'-triacetyl-8α-bromo-FMN (II) only at the starting pH 6.7, with acetyl groups being simultaneously hydrolyzed in positions 2', 3', and 4'. It should be noted that the analogical hydrolysis of 2',3',4',5'-tetraacetyl-8α-bromoriboflavin has been carried out by simple heating in water (13). From the disodium salt of the compound (III) 8α-hydroxy-FMN (III) has been obtained on the strong acidic cationite KU-2 (in H$^+$ form).

8α-Hydroxy-FMN (III) has been acylated with Ac$_2$O in pyridine; the etherification has been accomplished by simultaneous cyclization of the phosphate group to form 2',3'-diacetyl-8α-acetoxyriboflavin-4',5'-cyclophosphate (IV). This reaction is analogous to the preparation of 2',3'-diacetylriboflavin-4',5'-cyclophosphate under the same conditions (15).

In NMR spectrum of compound (IV) (D$_2$O), the signal of the 8-CH$_3$ group (2.57 ppm) is absent and the new signal of the 8-CH$_2$ group (5.62 ppm) appears. The IR spectrum of 2',3'-diacetyl-8α-acetoxyriboflavin-4',5'-cyclophosphate
shows an absorption band of aliphatic esters at 1100 cm\(^{-1}\) which probably may be attributed to the 8\(\alpha\)-acetyl group. From the examination of the IR spectrum of \(2', 3', 4', 5'\)-tetraacetylriboflavin it follows that the narrow absorption band of the acetoxy group in position 5' with low intensity at 1090 cm\(^{-1}\) is unlike acetoxy groups in positions 2', 3', and 4' with the broad intensive absorption band at 1050 cm\(^{-1}\) (Fig. 1).

8\(\alpha\)-Hydroxy-FMN (III) has the following UV and visible absorption spectra (in phosphate buffer pH 7.0), \(\lambda_{\text{max}}\), nm (\(\varepsilon\)): 223 (22000), 268 (16500), 365 (6500), 446 (5400). Unlike FMN, these spectra show characteristic hypsochromic shift (over 9 nm) of the second absorption band in the case of 8\(\alpha\)-substituted flavins (1-13) (Fig. 2). Usually the phosphate formation at the primary 5'-hydroxy group of the ribityl chain doesn’t change (or almost doesn’t change) the pattern of absorption spectra of riboflavin derivatives. Thus, it is interesting to point out the differences between the extinction ratios of the first and second maxima: for 8\(\alpha\)-hydroxyriboflavin (13) the value \(\varepsilon_{445}/\varepsilon_{363}\) is 1.28, while for 8\(\alpha\)-hydroxy-FMN this value is essentially smaller (\(\varepsilon_{446}/\varepsilon_{365}\) 0.83).

It is important to note that the reduction of the extinction value of the first absorption maximum, unlike the second absorption maximum, is characteristic for all synthesized compounds (compounds II, III and IV). As has been noted earlier (2, 6, 11) the substituents in position 8\(\alpha\) influence the fluorescence of flavins. While the fluorescence intensity of riboflavin and 8\(\alpha\)-hydroxyriboflavin are almost equal, in the case of 8\(\alpha\)-hydroxy-FMN (III), unlike FMN, the fluorescence is quenched over about 60% (Fig. 3). The similar quenching of fluorescence has
taken place for 2', 3'-diacetyl-8α-acetoxyriboflavin-4', 5'-cyclophosphate in comparison with 2', 3'-diacetylriboflavin-4', 5'-cyclophosphate (over about 50%).

![Fluorescence spectra](image)

Fig. 3. The fluorescence spectra (phosphate buffer, pH 7): 1. riboflavin; 2. 8α-hydroxyriboflavin; 3. FMN; 4. 8α-hydroxy-FMN; 5. 2', 3'-diacetylriboflavin-4', 5'-cyclophosphate. 6. 2', 3'-diacetyl-8α-acetoxyriboflavin-4', 5'-cyclophosphate.

We have studied the fluorescence of the 8α-hydroxy-FMN (III) versus pH. Analogously to FMN, it is unchanged within the region pH 2.5–8.5 and it is similar to the pH-dependence of fluorescence of riboflavin, 8α-hydroxyriboflavin and FMN, however, unlike FMN, its intensity is essentially lower (Fig. 4). Both the

![pH dependence of fluorescence](image)

Fig. 4. pH dependence of fluorescence: 1. riboflavin; 2. 8α-hydroxyriboflavin; 3. FMN; 4. 8α-hydroxy-FMN.
reduction of extinction of the first absorption maxima and the fluorescence quenching may serve as an evidence of the phosphate group effect upon the chromophore flavin molecular system in the presence of the 8α-hydroxy group.

There are two reflection points on the potentiometric curve during the titration of 8α-hydroxy-FMN (III) by 0.05 N NaOH that correspond to pK1 4.5 and pK2 8.4 and, therefore, to the formation of mono- and disodium salts of this compound. Potentiometric titration of 2', 3'-diacetyl-8α-acetoxyriboflavin-4', 5'-cyclophosphate (IV) has revealed a reflection point at pH 6.7 corresponding to cyclophosphate (Fig. 5).

8α-Hydroxy-FMN (III), as well as FMN, is transformed to corresponding 4', 5'-cyclophosphate by the treatment of N, N-dicyclohexilcarbodiimide in pyridine by the method (16).

EXPERIMENTAL

UV and visible spectra were determined in phosphate buffer, pH 7. NMR spectra were recorded on a Perkin-Elmer-20A spectrometer with 60 MHz in CD3OD or D2O. Fluorescence spectra were determined with an Hitachi-MPF-2A spectrofluorometer in phosphate buffer, pH 7.

2', 3', 4'-Triacetyl-FMN (I) was obtained from FMN according to the method (17) in perchlorate form. λmax, nm (ε): 224 (26500), 268 (28000), 374 (8900), 446 (10900). ε374/ε224 1.06; ε446/ε374 1.22. NMR (CD2OD): δ=8.01 (6-H), 7.75 (9-H), 2.60 (7-CH3), 2.45 (8-CH3), 1.70, 2.02 and 2.22 (Ac-groups). Found: C 39.91, H 3.99, N 8.46, P 4.02, Cl 4.78 %. C23H27N4O12P·HClO4. Calculated: C 40.45, H 4.13, N 8.20, P 4.53, Cl 5.19 %.

2', 3', 4'-Triacetyl-8α-bromo-FMN (II). 0.4 g of compound (I) were boiled
in dioxane with dibenzoyl peroxide for 45 min. The solvent was distilled off under reduced pressure, the oily residue was mixed with dry ether until the precipitate and dried in vacuo (P\textsubscript{2}O\textsubscript{5}): yield 0.35 g of compound (II). \(\lambda_{\text{max}}\) nm (\(\varepsilon\)): 224 (24800), 268 (25800), 377 (9300). \(\varepsilon_{268}/\varepsilon_{224} 1.04; \varepsilon_{446}/\varepsilon_{377} 0.89\). NMR (CD\textsubscript{3}OD): \(\delta=8.15\) (6-H), 7.92 (9-H), 2.62 (7-CH\textsubscript{3}), 3.65 (8-CH\textsubscript{2}), 1.70, 2.05 and 2.22 (Ac-groups). Found: C 41.24, H 4.70, N 8.26, Br 12.02, P 4.98%. \(\text{C}_{23}\text{H}_{26}\text{N}_{4}\text{O}_{12}\text{PBr}\). Calculated: C 41.76, H 4.01, N 8.47, Br 12.08, P 4.68.

8\(\alpha\)-Hydroxy-FMN (III). Aqueous solution of 3.0 g of compound (II) (pH 6.7) were heated at 65–70°C; at the end of the reaction the pH was 8.8–8.9. The solution was evaporated and under fractional precipitation by methanol 1.8 g disodium salt of compound (III) was obtained which was reprecipitated from water by addition of 96% ethanol; the product was dissolved in water and treated by cationite KU-2 (in H\textsuperscript{+} form) to give a solution at pH 2.5. The cationite was filtered, the filtrate was evaporated to produce an oily residue which was washed with ethanol and mixed with ether. After the drying in vacuo, 0.73 g of compound (III) was obtained. Found: C 43.50, H 4.48, N 11.48, P 6.10%. \(\text{C}_{17}\text{H}_{21}\text{N}_{4}\text{O}_{10}\text{P}\). Calculated: C 43.22, H 4.48, N 11.86, P 6.55%.

2',3'-Diacetyl-8\(\alpha\)-acetoxyriboflavin-4', 5'-cyclophosphate (IV). 0.26 g of compound (III) was acylated with 9 ml Ac\textsubscript{2}O in pyridine for 12 hr at 50°C. The solution was evaporated under reduced pressure and then several times with the addition of water and methanol. The residue was dissolved in ethanol and after twofold reprecipitation by the ether, 0.27 g of compound (IV) was obtained. \(\lambda_{\text{max}}\) nm (\(\varepsilon\)): 223 (22000), 268 (16800), 369 (5300), 446 (4600); \(\varepsilon_{268}/\varepsilon_{223} 0.76; \varepsilon_{446}/\varepsilon_{369} 0.87\). NMR (D\textsubscript{2}O): \(\delta=8.01\) (9-H), 7.95 (6-H), 5.62 (8-CH\textsubscript{2}), 2.35 (7-CH\textsubscript{3}), 2.28, 2.21 and 1.79 (Ac-groups). Found: C 48.00, H 4.48, N 9.41, P 5.57%. \(\text{C}_{23}\text{H}_{25}\text{N}_{4}\text{O}_{12}\text{P}\). Calculated: C 47.60, H 4.54, N 9.60, P 5.33%.

REFERENCES


