ADVANCES IN THE CHEMISTRY OF NATURAL FLAVINS

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The review discusses advances in the chemistry of isoalloxazines in the last 10 years, including 1978, in the field of the detection of new derivatives of flavin (7,8-dimethylisoalloxazine) and modifications of it in natural sources — microorganisms, fungi, and plant and animal tissues — and their isolation from these sources. Questions of the establishment of structure, chemical and physicochemical properties, chemical synthesis, and the biological activity of the new flavin vitamins and prosthetic groups of flavoproteins are considered. The priority of Soviet scientists in the discovery of the covalent type of bond with the prosthetic group in some flavin enzymes is noted.

The advances in the chemistry of the flavins in the last 10 years are due to the intensive and far-reaching development of the chemistry of compounds of the alloxazine and isoalloxazine series and to great achievements in this field — the detection in nature and isolation of numerous new biologically active natural flavins, and the determination of their structures, their synthesis, and the study of their chemical and physicochemical properties. The present review is devoted to this question.

The heterocyclic pyrimido[4,5-b]quinoxaline or benzo[g]pteridine condensed system forms the alloxazine and isoalloxazine series; the 7,8-dimethyl-10-N-substituted isoalloxazines (flavins) include natural compounds. The specific structure of isoalloxazine effects the biocatalytic transfer of an electron and a proton in key redox enzymatic reactions of the metabolism. In the form of riboflavin (vitamin B_2) and its nucleotide coenzymes — FMN and FAD — and also their modifications, the isoalloxazine structure is present in numerous enzymes that fulfill the biocatalytic function of the transfer of electrons and protons from NAD(P)-dependent enzymes or other donors directly, or via a chain involving a number of other carriers, to molecular oxygen. This function in metabolic processes is connected with the cleavage and synthesis of proteins, carbohydrates, and fatty acids, and with the liberation of energy. The flavin prosthetic group is bound covalently or less strongly to specific proteins, forming numerous flavin enzymes (flavoproteins).

The first yellow flavin pigment "lactochrome" was found in cows' milk serum by Blyth in 1879 [1]. Forty years later, a crude yellow water-soluble factor was isolated from milk [2], and in 1933 a yellow water-soluble flavin with a yellow-green fluorescence was isolated in the crystalline form: "lactoflavin" from cow' milk serum [3, 4], "ovoflavin" from egg yolk [5], and "lyochrom" from animal tissues [6], from malt and dandelion flowers [7], from liver [8], etc. Kuhn et al. [3-5] and others considered the structure of "lactoflavin" in detail. In 1935, on the basis of the results of a study of cleavage products, Karrer, Schöpp, Benz, Euler, Malmberg, Becker, and Frei [9, 10], and Kuhn, Reinemund, Weygand, and Ströbels [11] performed the first synthesis of lactoflavin — riboflavin (I) — which definitively determined its structure.

Riboflavin is widely used in therapeutic medicine and for the vitaminization of food and fodder products. The physiological requirement of a man for riboflavin as an essential nutritional factor is about 1 g per year. The esters of riboflavin, as preparations with a prolonged vitamin B_2 action, are of independent physiological importance; they stimulate the carbohydrate and lipid metabolism. The most interesting esters include: 2',3',4',5'-tetrabutyrylriboflavin (it also exhibits an antisclerotic effect) [12, 13], mixed esters of the reduced isoalloxazine form — 5-acetyl-2',3',4',5'-tetrabenzoyl-1,5-dihydroriboflavin [14] — and others. Some riboflavin derivatives and compounds of the alloxine series have exhibited anti-

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bacterial activity [15]. The biological activities of riboflavin analogs have been discussed in a review [16].

Simultaneously with the discovery and establishment of the structure of riboflavin its coenzyme forms were discovered. In 1932, Banga and Szent-Györgi [17] isolated the yellow coenzyme "cytoflav," which subsequently proved to be a phosphate of riboflavin, from porcine cardiac muscle. In the same year, Warburg and Christian discovered in and isolated from bottom-fermentation yeasts the first flavoprotein [18] which was subsequently called the "old yellow enzyme;" they subjected this enzyme to cleavage into protein and a fluorescing yellow pigment (riboflavin) [19]. As the prosthetic chromophoric group of the "old yellow enzyme'" the dehydrogenase of reduced NADP, Theorell isolated a flavin containining one molecule of phosphate — flavin mononucleotide (FMN) [20]; the prosthetic group was riboflavin [21]. In 1935, Kuhn and Rudy [22] performed the first synthesis of FMN (II) and in the subsequent year they definitively established its structure as riboflavin 5'-phosphate [23].

A third natural flavin P^1-(riboflavin-5')-P^2(adenosine-5') diphosphate, flavin adenine dinucleotide (FAD) (III) was isolated as a cofactor of D-(amino acid) oxidase from ovine kidney, equine kidney and liver [24], and yeast [25] in 1938; its structure was soon established (1939-40) [26, 27], and in 1952 Christie, Kenner, and Todd synthesized FAD [28].

FMN and FAD (Flavitan) are used in medicine as highly effective drugs in cases of hypovitaminosis B_2 and in the complex treatment of dystrophy of the retina, some dermatitises, acne rosacea, and other diseases.

No new natural flavins were discovered in the following 30 years, since it was assumed that only these two readily hydrolyzable prosthetic groups — FMN and FAD, riboflavin derivatives — were present in the numerous flavoproteins isolated from animal and plant tissues. However, facts have gradually accumulated concerning the participation in enzymatic reactions of flavins of undetermined structure and a wave of biological, biochemical, and chemical investigations has followed as a result of which, beginning at the end of the sixties, several new electron-transferring biocatalytic factors with an isoalloxazine structure containing various functional groups — modifications of riboflavin, FMN, and FAD in positions 3, 5', 6, 8, and 8a — have been discovered in neutral sources. At the present time, the number of natural flavins, flavin vitamins, and their coenzymes has risen from 3 to 22 (see formulas I-XXII).

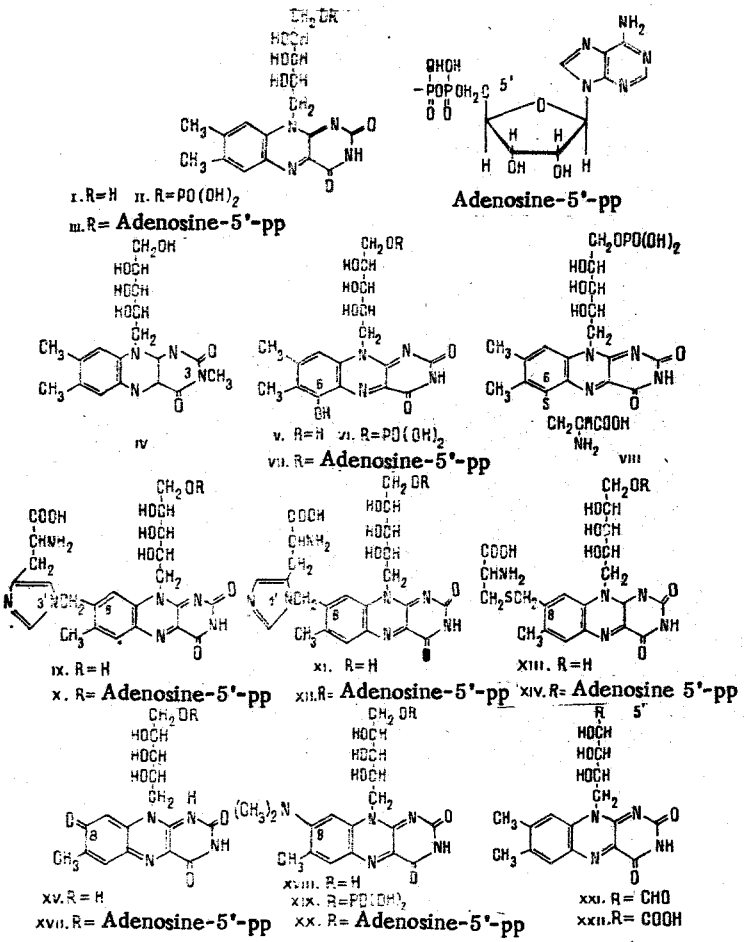
The new flavins are, from the structural point of view:

a) substituted riboflavins —
   at the pyrimidine ring in the 3(N)position (IV),
   in the benzene ring in position 6 (V-VIII),
   at the methyl group in position 8 (IX-XIV); and

b) modifications of riboflavin —
   at position 8, with the elimination of the 8-CH_3 group (XV-XX), and
   in position 5', with the oxidation of the hydroxymethyl group (XXI, XXII).

A flavin with a blocked cyclic imino group delimiting the system of binding with protein — N^3-methylriboflavin (IV) — has been isolated from the poisonous fungus *Panellus serotinus* [29]. The mixture of two yellow substances isolated from the fungus was acetylated and one product proved to be 2',3',4',5'-tetraacetylriboflavin. The second acetate, according to its PMR spectrum (M^+ m/e 558), and electronic absorption spectrum with a long wave λ_{max} at 445 nm similar to that of riboflavin, and independent synthesis, proved to be a riboflavin derivative methylated in position 3. Its acid hydrolysis gave a natural flavin which was identified as N^3-methylriboflavin (IV). N^3-Methylriboflavin (IV) was obtained synthetically by condensing 6-aminophenyl-3,4-dimethyl-D-ribitylamine with monomethylalloxan as early as 1935 by Kuhn et al. [11].

The new natural flavin vitamins and readily hydrolyzed flavin prosthetic groups include: 6-hydroxyriboflavin (V) and its coenzymes — 6-hydroxy-FMN (VI) from porcine liver glycolate oxidase, and 6-hydroxy-FAD (VII) from the electron-transferring flavoprotein of *Peptostreptococcus elsdenii*. As compared with riboflavin, FMN, and FAD, this type of covalently bound natural flavins has an additional substituent in position 6 by which a peptide bond to the apoprotein is effected. It must be noted that the hydrogen atom in this position of the isoalloxazine molecule is relatively immobile, unlike the reactivities of positions 7, 8, and 9 of the benzene ring in relation to the electrophilic substitution.



From the electron-transferring flavoprotein of *Peptostreptococcus elsdenii*, in addition to the orange chromophore 8-hydroxynor-FAD (XVII) (see below), in 1974 FAD (III) and a green chromophore — 6-hydroxy-FAD (VII) — were isolated, and from the glycolate oxidase (E.C. 1.1.3.1) from porcine liver 6-hydroxy-FMN (VI) was isolated [30]. Information on the presence in glycolate oxidase of, in addition to FMN, a new green flavin chromophore had been obtained previously [31]. The structure of 6-hydroxyflavin was suggested on the basis of its capacity for complex-formation with metals, which was analogous to the capacity of model 6-hydroxyflavins that had been synthesized previously, and on the basis of the other facts given by Schöllnhammer and Hemmerich [32]. In aqueous solution, 6-hydroxy-FAD (VII) changes color from (grass) green to yellow between pH 9 and pH 5. Its absorption spectrum at pH 9 has λ_{max} ~260, ~427, and 600 nm, and at pH 5 ~ 264 and 422 nm [30]. At intermediate pH values, a smooth transition of the absorption bands is observed, the changes corresponding to a pK of 7.1 and showing isobestic points with λ_{max} 263, 299, 354, 413, 477, and 507 nm, which made it possible to suggest the ionization of a very weak acid [30]. Synthetic model 6- and 9-hydroxyflavones have been obtained in an acid medium by the photo-addition of water, alcohols, or carboxylic acids to the flavin nucleus [32, 33] with the simultaneous intramolecular migration of a hydrogen atom from the position of attachment to the 5-N atom and subsequent oxidation of the 6- or 9-hydroxy-1,5-dihydroflavin obtained [32].

The spectral properties of the 6- and 9-hydroxyisoalloxazines do not differ appreciably [32]. Thus, these compounds show a characteristic absorption band with λ_{max} 405 nm. The hydroxyl groups both in position 6 and in position 9 show a relatively low pK_a value (about 7) [33]. The synthetic hydroxylated compounds do not fluoresce. A phenolic oxygen in position 6 of the isoalloxazine molecule greatly increases the reactivity in relation to the formation of complexes with metals. The green chromophores from glycolate oxidase and the electron-transferring flavoprotein (VI and VII) form complexes with $Cu^{(II)}$ in an acetate buffer at pH 6 with the participation of the nitrogen atom in position 5, which is connected with a bathochromic shift of the long-wave absorption band approximately from 418 to 448 nm at pH 6 and with an increase in the absorption of the band with a maximum at about 320 nm, apparently showing the presence of a hydroxy group in position 6 of the isoalloxazine molecule [30]. The 9-hydroxyflavins do not give complexes with metals [32, 33].

Some other characteristics of the PMR and ESR spectra have also been obtained which are in favor of the idea of the structure of the new flavins as 6-hydroxy derivatives [32]. However, no proof of the structure of 6-hydroxy-FMN and 6-hydroxy-FAD by cleavage to the corresponding substituted alloxazines, by the determination of a D-ribityl group in position 10, or by direct synthesis has been given.

In many flavoproteins, the mono- and dinucleotides FMN and FAD are connected with the apoenzyme by a readily hydrolyzable bond. The same hydrolyzable bond of the prosthetic group with the protein moiety of the enzyme is also characteristic for the new flavin coenzymes — 6-hydroxy-FMN (VI), 6-hydroxy-FAD (VII), and 8-hydroxynor-FAD (XVII).

Another type of bond between an isoalloxazine coenzyme molecule and the protein moiety of the enzyme is also known. In 1953-1955, Povolotskaya and Bukin [34-36] discovered the presence in tissues of a flavin covalently bound to a protein. In 1953, Povolotskaya (Bukin's laboratory of the Institute of Biochemistry of the Academy of Sciences of the USSR) in a paper on "A new form of riboflavin bound to protein" [34] reported the establishment of the existence in animal and plant tissues (and, particularly, in the growth of seeds) of a new, previously unknown, form of the bond of a flavin with a protein which was resistant to acid hydrolysis but was cleaved by proteolytic enzymes of the trypsin type under weaklyalkaline conditions; the reaction yielded a flavin molecule in the form of a dinucleotide. It was concluded that the flavin adenine dinucleotide was attached to the protein through a peptide bond which was cleaved on proteolysis. The dinucleotide form was then subjected to cleavage with phosphatases or with trichloroacetic acid to free flavin, which was determined quantitatively.

It was found that the succinate dehydrogenase of animal tissues — calf cardiac muscle and pigeon thoracic muscle [35, 36], and also bovine [37, 38] and porcine [39] cardiac muscle was not identical with FMN and FAD [35-37, 40] but included in its molecule a new prosthetic flavin group — a dinucleotide coenzyme of the FAD type — the adenine dinucleotide form of riboflavin firmly (covalently) bound to protein [35, 36]. These facts [35-37, 40] showed the flavin nature of succinate dehydrogenase, which had previously been disputed. The dinucleotide nature of the cofactor of succinate dehydrogenase was established by spectroscopic and chromatographic methods, and also by a test with D-(amino acid) oxidase [35, 36, 40, 41]. This exceptionally important discovery [34-36] found further development in other investigations. In 1960, Kearney [42] established that the cofactor of succinate dehydrogenase of the FAD type was covalently bound to one of the amino acids of the protein, and subsequent investigations, over 10 years, led to the establishment of the structure of the covalently bound flavocoenzyme of succinate dehydrogenase [43, 44]. It follows from the results obtained originally, that in natural materials covalently bound flavins are present in considerable amount, in even larger amount than the acid-hydrolyzable flavins. It is possible that succinate dehydrogenase is the main source of covalently bound flavins [35, 36].

One of the natural covalently bound flavins substituted in position 6 by a mercapto amino acid (cysteine) has been found in the form of a coenzyme — 6-S-cysteinyl-FMN (VIII) — in trimethylamine dehydrogenase. 6-S-Cysteinyl-FMN [45] contains phosphorus in the pentitol side chain and a S-(amino acid) in a position other than 8α [46]. The flavin (VIII) does not possess fluorescence and is extremely photolabile; in the form of a prosthetic group it is contained in bacterial trimethylamine dehydrogenase, catalyzing the oxidative demethylation of trimethylamine to dimethylamine and formaldehyde [46].

Because of the selective mobility of the hydrogen atom of the methyl group in position 8 of riboflavin, substitution by an amino acid (containing an imidazole ring or a SH group) takes place and a C–N bond at the 1-N or 3-N position of the histidine ring or a C–S bond through the sulfide group of a cysteine residue arises. Such covalently bound 8α-(amino acid) derivatives of isoalloxazine — new flavin vitamins and coenzymes — include: 8α-(histidin-3-yl)riboflavin (IX); its coenzyme, 8α-(histidin-3-yl)FAD (X) from succinate dehydrogenase, D-6-hydroxynicotine oxidase, and sarcosine dehydrogenase; 8α-(histidin-1-yl)riboflavin (XI); its coenzyme, 8α-(histidin-1-yl)-FAD (XII) from thiamine dehydrogenase, β-cyclopiazonate oxidocyclase, L-gulonolactone oxidase, D-gluconolactone dehydrogenase, and cholesterol oxidase; 8α-cystein-S-yl-riboflavin (XIII); and its coenzyme, 8α-cystein-S-yl-FAD (XIV) from the monoamine oxidase from Chromatium cytochrome c-552 and *Chlorobium thiosulfatophilum* c-553.

The prosthetic group of succinate dehydrogenase (E.C. 1.3.99.1) is 8α-(histidin-3-yl)-flavin (IX) [43, 44], which, in the form of 8α-(histidin-3-yl)-FAD (X), is present in the active center of the succinate dehydrogenase from the mitochondria of bovine cardiac muscle [47]

Succinate dehydrogenase catalyzes the dehydrogenation of succinic acid to fumaric acid in the tricarboxylic acid metabolic cycle, which is the main source of the generation of energy in the macroorganism.

By cleavage with proteolytic enzymes (trypsin, chymotrypsin) [34], succinate dehydrogenase (SD) yielded a flavin of the FAD type with the absorption spectrum λ_{max} 267, 360, and 450 nm, including an AMP molecule [41]. Then a flavin-peptide containing a pyrophosphate bond, adenylic acid, and D-ribose was isolated [42]; this SD-FAD differed from FAD by a hypsochromic shift of the second absorption band from 375 to 350 nm, and also by other properties. On brief hydrolysis in 1 N hydrochloric acid at 100°C, SD-FAD yielded SD-FMN and AMP, and in 6 N HCl at 95°C for 10 h it gave SD-riboflavin containing a ribityl substituent in position 10, the nature of which was established with the aid of periodate oxidation. As was found, the molecule of this compound also includes 6-7 amino acids: alanine, serine (2 residues), glutamic acid, valine, and threonine [42]. The partial hydrolytic cleavage of the succinate dehydrogenase from animal tissues with trypsin at the peptide bonds led to a flavin peptide with 23 amino acids [48], and stronger hydrolysis with trypsin and chymotrypsin led to the formation of a flavin pentapeptide.

The complete cleavage of SD at the peptide bonds (6 N HCl, 95°C) gave 8α-(histidin-3-yl)-riboflavin (IX) [43]. Its absorption spectra in water at pH 7 was: λ_{max} 219, 268, 355, 445 nm, the maximum of the second absorption band having a characteristic shortwave shift as compared with riboflavin (λ_{max} 372 nm) by 17 nm [43]. It was subsequently found that the shortwave shift of the second absorption band is a distinguishing feature of all 8α-substituted flavins [50]. Riboflavin fluoresces with λ_{fl} of about 520 nm with almost the same intensity in the pH range of 3-9 and the photo-activation with UV light of compound (IX) in water led to the same yellow-green fluorescence, but its intensity depended on the pH and was extinguished according to a curve corresponding to a pH value of 4.4 [43, 51].

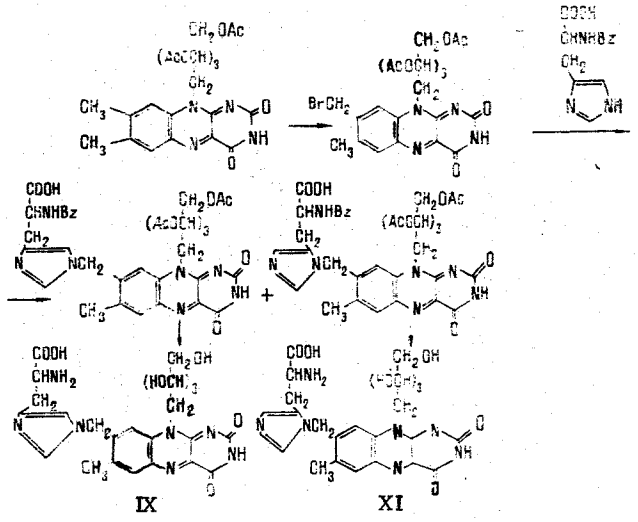
In the establishment of the structure of 8α-(histidin-3-yl)riboflavin (IX), it was found that the position of binding of the flavin peptide in SD cannot be position 1, 2, 3, 4, 5, 6, or 10 of the isoalloxazine ring. Thus, the alkaline hydrolysis of SD-FAD forms urea, which excludes substitution in positions 1 and 3 [52]. The substitution by the amino acid of position 10 must also be excluded, since on alkaline hydrolysis the ribityl side chain is split out and the bond with the amino acid is not cleaved [42]. With various substituents — alkyl, aryl, amino, and mercapto groups — in positions 2, 4, and 5 a reversible reaction takes place under conditions of weak oxidation or hydrolysis [53, 54]. In its ESR spectrum, the cation radical of the flavin (IX) gives a hyperfine structure with a decrease in the width of the signal to 46 G as compared with the 52 G of the cation radical of riboflavin [51]. Substitutions in positions 7 and 9 are excluded, since they are characterized by low spin densities [40], and substitution in position 6 is excluded because this would change the nature of the absorption spectrum as compared with that of riboflavin. The presence of a methylene group in position 8 of the flavin (IX) was found from its nuclear double resonance spectrum [55]. Thus, on the basis of the facts obtained, it was established that the peptide chain in SD is bound by a methylene group to the benzene ring of the flavin [51, 56]. Severe acid hydrolysis of the SD-riboflavin in the absence of atmospheric oxygen, and also neutral photolysis and reductive cleavage led to histidine, as in the cleavage of 3-benzylhistidine [43].

On the basis of the results of a study of the properties of SD-flavins and of the complete synthesis of SD-riboflavin, in 1970 two groups of workers — Hemmerich, Ghisla, and Hartmann, and Singer and Walker — definitively established the structure of compound (IX) as 8α-histidinylriboflavin [43, 44], and consequently that of 8α-histidinyl-FAD (X), covalently bound by a peptide bond with the protein moiety of succinate dehydrogenase. The synthesis of 8α-histidinylriboflavin (IX) was based on the increased reactivity of the methyl group in position 8 of the isoalloxazine ring; this position is a center of low π-electron density. The 8-CH_3 group possesses acidic properties and exchanges its hydrogen atoms for deuterium [57]. It is capable of dimerization with the formation of a benzoquinoid bis-riboflavin even under physiological conditions of the medium [58]. Riboflavin 5'-diphosphate likewise undergoes oxidative dimerization under strongly dehydrating conditions (orthophosphoric acid and phosphorus pentoxide) [59]. The reactivity of the 8-CH_3 group is also shown in the interaction of lumiflavin with nitrous acid in boiling acetic acid, which leads to its oxidation to a carboxy group with the formation of 8-carboxynorlumiflavin [58]. Such increased reactivity of the methyl group in position 8 of a flavin is connected with its pronounced hyperconjugation, which distinguishes it from the 7-CH_3 group [51].

The synthesis of 8α-(histidin-N^3-yl)riboflavin (IX) [44] was performed by brominating 2',3',4',5'-tetraacetylriboflavin with the molecular bromine in the presence of benzoyl peroxide with heating in dioxane; the resulting 2',3',4',5'-tetraacetyl-8α-bromoriboflavin, on reaction with N_α-benzoylhistidine in anhydrous dimethylformamide with the subsequent acid hydrolysis of the protective groups [44, 47] led to a mixture of two isomers — 8α-(histidin-3-yl)riboflavin (IX) with a yield of 75-80% and 8α-(histidin-1-yl)riboflavin (XI) [47, 60]; this mixture was separated by thin-layer chromatography on cellulose or by paper electrophoresis on paper in pyridine—acetate buffer [47]. It was accurately established that SD-riboflavin is 8α-(histidin-3-yl)riboflavin [47] and, consequently, the position of binding of the methylene group of the isoalloxazine molecule is the nitrogen atom in position 3 of the imidazole ring of histidine [47].

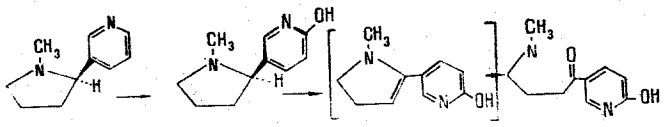
Synthetic 8α-(histidin-3-yl)riboflavin (IX) has an absorption spectrum with λ_{max} 221, 268, 355, and 445 nm [47]. For the ESR spectra of the cation radical of the flavin (IX) from SD and synthetic 8-substituted flavin cation radical [61] a decrease in the width of the tota signal from 52 (for riboflavin) to 46 G is observed, which gives grounds for connecting this common property with the presence of a substituent in position 8α [61].

As has recently been established 8α-(histidin-3-yl)-FAD (X) forms part of the structure of the succinate dehydrogenase from *Vibrio succinogenes* [62]. In addition, this flavin is the covalently bound prosthetic group of some other flavoproteins.



IX XI

The prosthetic group 8α-(histidin-3-yl)-FAD is attached by a peptide bond to protein in the D-6-hydroxynicotine oxidase isolated from *Arthrobacter oxidans* [63]. D-6-hydroxynicotine oxidase catalyzes the oxidation of D-6-hydroxynicotine; this reaction is an intermediate step in the enzymatic oxidative cleavage of the alkaloid d,*l*-nicotine to 6-hydroxypyridin-3-yl 3-(methylamino)propyl ketone,which takes place on the combined presence of nicotine dehydrogenase and ketone dehydrogenase. L-6-Hydroxynicotine is oxidized by L-6-hydroxynicotine oxidase containing noncovalently bound FAD.



As the result of the proteolytic cleavage of D-6-hydroxynicotine oxidase, a flavin peptide was isolated with an established amino acid sequence [65]:

FAD-(3-N)His<Asp-Pro-Asn-Asn-Gly-Gly-Ser
<Tyr-Gln-Pro-Ala

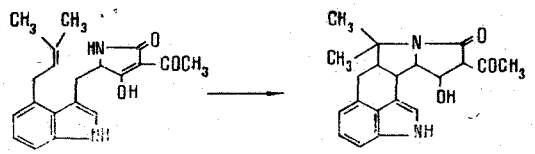
It contains the flavin in the dinucleotide form [66]. Acid hydrolysis of the flavin-peptide yielded a flavin with an absorption spectrum of 268, 346, and 446 nm; the second absorption band has a hypsochromic shift in comparison with the spectrum of riboflavin. The fluorescence of the flavin peptide is quenched according to a curve corresponding to a pK value of 4.65. Hydrolysis with strong acids cleaves the flavin to give histidine. From its physico-

chemical properties, and also the results of thin-layer chromatography, the structure of this flavin was established as 8α-(histidin-3-yl)riboflavin; it was identical with the synthetic flavin (IX) from succinate dehydrogenase [66]. The covalently bound prosthetic group of sarcosine dehydrogenase from rat liver mitochondria [67] and a strain of Pseudomonas [68] is 8α-(histidin-3-yl)-FAD (X) [69]. On cleavage with trypsin-chymotrypsin, the flavoprotein yielded the flavin in the form of the dinucleotide [67, 68]. Under the action of aminopeptidase M, bacterial sarcosine dehydrogenase gives a flavin-peptide [69]:

$$\text{Fl-(3-N) His} \begin{cases} \text{Gly-Glu-Ser-Lys-Asp} \\ \text{(Asp, Ala, Thr)} \end{cases},$$

which at the level of the FMN showed a hypsochromic shift of the maximum of the second absorption band that is characteristic for 8α-substituted flavins, and also a pK value for the quenching of fluorescence of 4.8; furthermore, on cleavage the methylated protein yielded 1-methylhistidine [69].

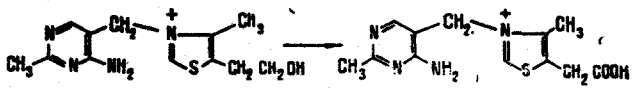
Some flavin 8α derivatives are attached to histidine at N^1 of the imidazole ring, and not N^3, as in succinate dehydrogenase. The prosthetic group of 8α-(histidine-N^1-yl)-FAD (XII) has been isolated from the β-cyclopiazonate oxidocyclase of *Penicillium cyclopium* [70], which catalyzes the dehydrogenation and cyclization of β-cyclopiazonic acid to α-cyclopiazonic acid.



The flavoprotein molecule includes the flavin in the dinucleotide form [72, 73]. The cleavage of β-cyclopiazonate oxidocyclase with trypsin and chymotrypsin formed a flavin-peptide which at pH 7.2 has λ_{max} 354 and 447 nm [73]; the hypsochromic shift of the second absorption band (as compared with FMN) is connected with 8α-substitution. The fluorescence excitation spectrum has a hypsochromic shift of the FMN band from 372 to 348 nm at pH 3.4 [70]. Intensive quenching of fluorescence takes place with pK 5.4 [70], while pK for the quenching of the fluorescence of 8α-(histidin-N^1-yl)flavins is 4.4 [43, 51]. The flavin obtained by acid hydrolysis of the flavin-peptide has the structure of 8α-(histidin-N^1-yl)riboflavin (IX), as was shown by comparing its chemical and physicochemical properties with those of the synthetic flavin (XI).

Synthetic 8α-(histidin-N^1-yl)riboflavin (XI) was obtained with a yield of 20-25% as a byproduct of the synthesis of 8α-(histidin-N^3-yl)riboflavin (IX) by the reaction of 2',3',4',5'-tetraacetyl-8α-bromoriboflavin with Nα-benzoylhistidine followd by acid hydrolysis and the separation of the two flavins formed by high-voltage electrophoresis [47, 60, 74]. It was completely identical with the flavin (XI) from β-cyclopiazonate oxidocyclase and thiamine dehydrogenase in its absorption spectrum, the pK value of 5.2 for the quenching of fluorescence, electrophoretic mobility, and reduction with sodium tetrahydroborate [60]. The reductive cleavage of the synthetic 8α-(histidin-1-yl)riboflavin methylated in the imidazole ring led to 3-methylhistidine [60, 75], while the methylated 8α-(histidin-3-yl)riboflavin gave 1-methylhistidine.

In the form of a covalently bound coenzyme, 8α-(histidin-1-yl)-FAD (XII) is also present in the thiamine dehydrogenase of soil bacteria [60, 72, 76]. This flavoprotein catalyzes the four-electron oxidation of the ethyl group of thiamine; the reaction leads to the formation of thiamine acetic acid [76-78]:



Thiamine dehydrogenase has an absorption spectrum with λ_{max} 368 and 444 nm [77], and the flavin of its prosthetic group is of a dinucleotide nature [72]. The cleavage of the flavoprotein with trypsin and chymotrypsin formed an acid-soluble flavin-peptide which at the level of the FMN reveals the quenching of fluorescence with pK 5.8 (considerably higher than for the analogous flavin from SD); the quenching of the fluorescence apparently arises because the methyl group of the isoalloxazine has as substituent an imidazole ring bound through a

nitrogen atom. The second fluorescence excitation maximum has a hypsochromic shift to 345 n at pH 3.4 (in comparison with FMN). This shows that the flavin-peptide is substituted in position 8α [76].

An (amino acid)-substituted flavin has been obtained by the acid hydrolysis of the flav peptide; on subsequent severe acid hydrolysis, one mole of histidine (substituting the 8α po sition) [76] and 8-formylnorflavin was obtained in contrast to 8α-(histidin-3-yl)flavin, whi under the same conditions, forms 8α-hydroxynorflavin [76]. On the basis of the facts obtaine the formation of 3-methylhistidine in the cleavage of methylated flavin, which is analogous to the chemical behavior of the synthetic compound (XI) (see above), it follows that in the prosthetic group of thiamine dehydrogenase the histidine is bound to the flavin molecule through the nitrogen atom in position 1 [74, 75].

8α-(Histidin-1-yl)-FAD (XII) also forms the covalently bound prosthetic group of L-gulo nolactone oxidase (E.C. 1.1.3.8) of rat liver [79, 80]. This enzyme catalyzes the conversion of L-gulono-γ-lactone to L-ascorbic acid in the last stage of its biosynthesis. The absorption spectrum of the purified pigment at the FMN level has λ_{max} 265, 346, and 443 nm, and the pK value for the quenching of fluorescence is 5.8 [80].

8α-(Histidin-N^1-yl)-FAD (XII) is apparently a component of the prosthetic group of the D-gluconolactone dehydrogenase of *Penicillium cyaneo-fulvum* [81] and of the cholesterol oxidase (E.C. 1.1.3.6) of *Schizophyllum commune* [82]. It follows from this that the second absorption maximum of the flavin is at 350 nm [81] or 355 nm [82], i.e., it experiences a hypsochromic shift. Severe acid hydrolysis leads to the formation of histidine [81], and quench ing of fluorescence with pK 5.1 is observed [82].

Under the conditions of acid hydrolysis, intramolecular cyclization of the 8α-(histidin- N^1- and N^3-yl)riboflavins takes place with simultaneous dehydration and the formation of 2',5'-anhydroflavins [83].

8α-(Histidin-N^1- and -N^3-yl)-substituted neutral flavin radicals react with molecular oxygen 2-10 times more slowly than riboflavin; the values of their ionization constants are 1-1.5 pK_a units lower, which reflects the electron-accepting effect of the 8α substituents [84].

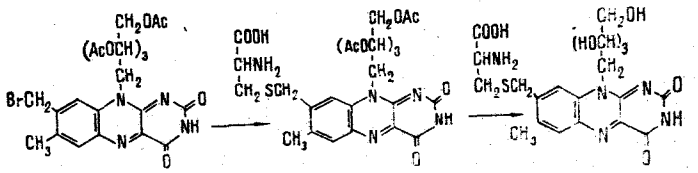
Some synthetic 8α-amino-substituted flavins, such as the 8α-(histidin-N-yl), 8α-(methionin-N-yl), 8α-(homoserin-N-yl), 8α-(glutamin-N-yl), and 8α-(γ-aminobutyryl) derivatives of 2',3',4',5'-tetraacetylriboflavin in aqueous solutions at high concentrations exhibit the properties of nonspecific inhibitors of riboflavin in *Bacillus subtilis* [85]. The 8α-(lysin- N^{ε}-yl) and 8α-(tyrosin-O-yl) [86] and the 8α-sulfonamide [15] derivatives of 2',3',4',5'-tetraacetylriboflavin have been synthesized [15]. The metabolism of 8α-(amino acid) derivatives of riboflavin has been studied in rats [87].

It has been established previously that the undissociated cofactor of monoamine oxidase from bovine kidney and liver is not FAD but a sulfur-containing derivative of it [88, 89]. As has been found, 8α-(cystein-S-yl)-FAD (XIV) is present in the active center of the monoamine oxidase of animal tissues — the mitochondria of bovine liver [90-93] and of porcine brain [94] — as prosthetic group [90-92, 95]. In its biochemical function, monoamine oxidase (E.C. 1.4.3.4) takes part in the catalytic deamination of monoamines; the selective inhibition of this enzyme in brain tissues is used for treating psychic diseases.

The stepwise acid hydrolysis of monoamine oxidase (MAO) has yielded "MAO-flavin" — a flavin-oligopeptide at the level of the mononucleotide FMN-(S)Cys$\begin{matrix}\nearrow\text{Gly-Gly-Ser}\\ \searrow\text{Tyr}\end{matrix}$, and then 8α-(cystein-S-yl)riboflavin (XIII) [90, 92]. The absorption spectrum of the flavin-oligopeptide has bands with λ_{max} 268, 367, 445 nm; here the maximum at 374 nm characteristic for FMN has a hypsochromic shift of 7 nm, like other 8α-substituted flavins [50]. The flavin (XIII) has the yellow-green fluorescence characteristic for FAD which, however, has a low yield over the whole pH range — only 10% of the fluorescence of riboflavin — but does not have even more pro nounced quenching in the pH range of 3.4-8.0 as is observed in the case of 8α-(histidin-N^3-yl)riboflavin [91]. The total width of the signal (44 G) in the ESR spectrum of the cation radical of the flavin-peptide is less than the total width of the signal (49 G) of riboflavin which may be considered as the result of the influence of the flavin substituent in position 8α [90]. It follows from the example of synthetic cystein-S-yl-riboflavin-peptides that the

substitution of the 8α position with a sulfide is accompanied by an increase in the redox postential by 0.025 V [96]; the thioester substituent of an 8α-S-flavin-peptide causes a pronounced quenching of fluorescence (about 98%) in aqueous solution, but in nonaqueous solvents the fluorescence rises 20-fold [97].

In 1971, Ghisla and Hemmerich [95] synthesized 8α-crystals-S-ylriboflavin (XIII) by condensing 2',3',4',5'-tetraacetyl-8α-bromoriboflavin with cysteine hydrochloride followed by hydrolysis.

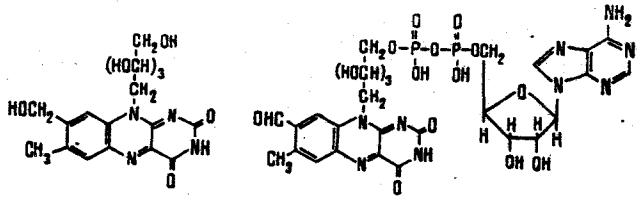


The flavin (XIII) has an absorption spectra at pH 7 with λ_{max} 268, 360, 445 nm, analogous to that of the natural flavin. The hypsochromic shift of the second absorption band is apparently connected with the repulsion of electron density by the substituent in the 8α position [95]. The sulfide substituent causes a pronounced quenching of fluorescence over the whole pH range (from 2 to 10) as compared with riboflavin. The ESR spectrum of the synthetic flavin (decrease in the total width of the signal with 52 G for riboflavin to 46 G) is similar to that of the natural flavin; it reflects the loss of ESR-active protons on the introduction of a substituent into the 8α position. The identity of the properties of the natural flavin-peptide and of the synthetic flavin (XIII) was established by Singer et al. [92].

8α-Cystein-S-yl-FAD (XIV) is also found in Chromatium cytochrome c-552 [98] and *Chlorobium thiosulfatophilum* cytochrome c-553 [99, 100]. The existence of a covalent bond of the flavin in the Chromatium cytochrome c-552 was established as early as 1971-1973 [101-105]. Two flavin-peptides have been isolated from Chromatium [106]: the peptic FAD-(S)Cys$\begin{matrix}\text{Thr-Tyr}\\ \text{Tyr}\end{matrix}$ and the tryptic FAD-(S)Cys$\begin{matrix}\text{Thr}\\ \text{Tyr}\end{matrix}$. On titration with sodium dithionate, the flavin-peptide from the Chromatium cytochrome c-552 adds two electrons, just like the 8α-cystein-S-ylriboflavin (XIV) [98]. For the flavocytochrome c-552 from Chromatium a thiosemiacetal structure in the form of 8α-cysteinyl-8α-hydroxyflavone was suggested previously [106, 107]. The erroneousness of such a structure was shown by Kenney and Singer [98].

The *Chlorobium thiosulfatophilum* cytochrome c-553 [100] contains a flavin in the dinucleotide form. A flavin-peptide has been isolated in which the sequence of amino acids surrounding the chromophore has been established [100]: Fl-(S)Cys$\begin{matrix}\text{Pro-Phe-Ser-Asn}\\ \text{Thr-Val}\end{matrix}$. At pH 5, the absorption spectrum of the flavin-peptide has maxims at 366 and 449 nm, the UV maximum having a hypsochromic shift as compared with the maximum of riboflavin. Acid hydrolysis forms cysteine and an 8-formylnorflavin. From this and other results, the flavin in the cytochrome apparently has the structure of 8α-cystein-S-yl-FAD (XIV).

There are grounds for assuming that 8α-hydroxy-FMN either is a metabolite or takes part in the biosynthesis of 8α-(amino acid) derivatives of flavins. 8α-Hydroxy-FMN has been synthesized by Zhilin and Berezovskii [108, 109] by heating 2',3',4'-triacetyl-8α-bromo-FMN in an aqueous solution of sodium bicarbonate. As compared with FMN, in the pH range of 2-9 the fluorescence of the 8-hydroxyflavin is 60% quenched [108, 109].



The synthesis of 8α-hydroxyriboflavin has also been derived [110]. The 8-hydroxy derivatives

of the flavins have a characteristic hypsochromic shift at the second maximum in the absorption spectrum by 9 nm in comparison with the 8α-unsubstituted flavins.

McCormick [111] synthesized 8-formylnor-FAD as a possible metabolite of the 8α-(amino acid) flavins. Riboflavin was first selectively brominated in the 8α position with bromine in a mixture of dioxane and pyridine, and the 8α,8α-dibromoriboflavin formed was converted by acid hydrolysis into 8-formylnorriboflavin. The 5'-hydroxy group of this compound forms a semiacetal with the formyl group in position 8 that is stable in a neutral or acid medium at room temperature [112]. By phosphorylation with phosphorochloridic acid, the 8-formylnorriboflavin was converted into 8-formylnor-FMN, which was condensed with the N',N"-dicyclohexylmorpholine-4-carboxyamidinium salt of AMP 5'-phosphoromorpholidate to give 8-formylnor-FAD. The UV spectrum of this flavin showed a hypsochromic shift of the second absorption maximum to 354 nm (as compared with 370 nm for FAD) [111].

As compared with riboflavin ($E_{m,7}$ — 0.190 V [113]), the 8α-substituted flavins have higher redox potentials (by 0.02-0.05 V); 8-hydroxyriboflavin has $E_{m,7}$ — 0.170 V, 8α-cystein-S-ylriboflavin (XIII) — 0.169 V, 8α-(histidin-N^3-yl)riboflavin (IX) — 0.160 V, and 8-formylnorriboflavin — 0.159 V [113]. At the same time, the direct replacement of a CH_3 group in position 8 by an amino group or chlorine considerably raises the redox potential: 8-aminonorriboflavin has E_0' — 0.066 and 8-chloronorriboflavin has E_0' — 0.055 V, in comparison with E_0' — 0.208 V for riboflavin at pH 7 [114]. These flavins have proved to be antimetabolites of riboflavin in testing on *Lactobacillus casei* [115].

In a study of the metabolism of 8α-(amino acid) derivatives of riboflavin such as 8α-(histidin-N^3-yl)riboflavin (IX) and 8α-(cystein-S-yl)riboflavin (XIII) in the form of its N-acetate on B_2-avitaminotic rats, it was found that on administration per os or intraperitoneally these compounds are assimilated, but they are not split to give riboflavin and, consequently, cannot serve as a source of vitamin B_2 [87].

New flavins attached to the protein of a flavoprotein by a readily hydrolyzable bond include: 8-hydroxynorriboflavin (XV); its coenzyme 8-hydroxynor-FAD (XVII) from NADH dehydrogenase; and an electron-transporting flavoprotein from *Peptostreptococcus elsdenii*.

A new orange flavin prosthetic group which is readily split off from the protein part of the molecule by heating or by extraction with trichloroacetic acid has been found in the NADH dehydrogenase (E.C. 1.6.99.3) from the obligate anaerobic bacterium *Peptostreptococcus elsdenii*; this prosthetic group is similar in its absorption and fluorescence spectra to FAD but is modified in the isoalloxazine nucleus [116]. This compound has proved to be a new noncovalently bound orange flavin, and its structure was established in 1973 by Ghisla and Mayhew [117] as 8-hydroxynor-FAD (XVII).

The dehydrogenase of reduced NAD catalyzes one of the key reactions of the biological oxidation of a substrate with, in the final account, the transfer of electrons and protons to an oxygen molecule; taking place simultaneously with this is the regeneration of the biocatalyst of the primary electron acceptor — nicotinamide adenine dinucleotide (NAD).

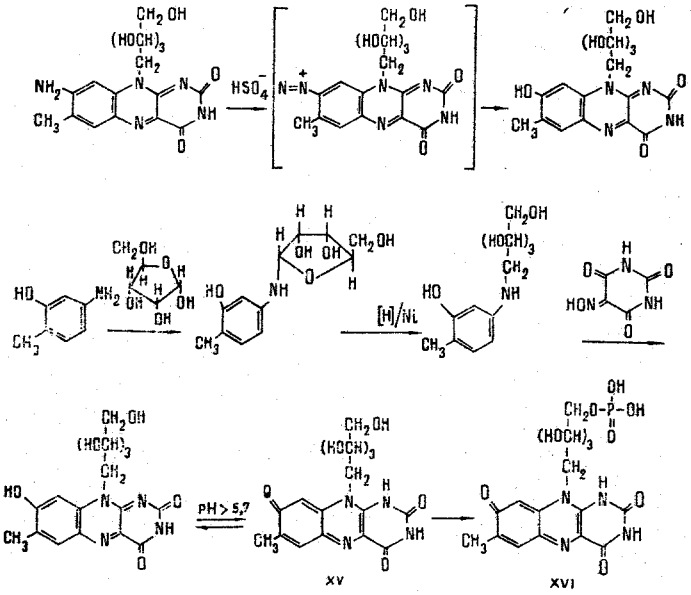
The new flavin (XVII) is bound to the apoenzyme of D-(amino acid) oxidase and to the apoprotein of the electron-transferring flavoprotein [118, 119]; consequently, it has a dinucleotide structure. This also follows from the fact that its cleavage with phosphodiesterase forms AMP [117] and a mononucleotide of the isoalloxazine series — "orange compound of FMN" (XVI), which binds to an apoflavodoxin from *P. elsdenii* [118, 119]. Hydrolysis of the phosphate group of this flavin forms a compound the properties of which are close to those of riboflavin. It follows from the PMR and ESR spectra that, in contrast to riboflavin, the isoalloxazine structure of the flavin (XVI) lacks a methyl group in position 8 [118]. The ionization constants of the new flavin — pK_a 0.7, 4.8, and 11.5 (in contrast to riboflavin, having pK_a 0 and 10.0 [120]) show the presence of an additional functional group which is conjugated with the chromophoris system of the isoalloxazine molecule [117]. The pK_a value of 4.8 for such a "phenolic" hydroxy group present in position 8 depends on its deprotonation. The state of ionization of 8-hydroxynorflavins, which can be represented by two tautomeric structural formulas is responsible for the unusual absorption spectrum of natural and synthetic model compounds with a fixed electronic structure [118] connected with protonation in positions 1 and 8. Such structures are characterized by a long-wave absorption maximum at 470 and 445 nm. In aqueous solution, the 8-hydroxy derivatives have a characteristic yellow-green fluorescence in UV light similar to that of ordinary flavins. As the result of a photolytic cleavage reaction at pH 3 with the partial splitting out of the substituent at N-10,

the "orange compound of FMN" (XVI) gives rise to a product which has been identified as 8-hydroxy-7-methylalloxazine [117]. However, the structure of the 8-hydroxynor-FAD (XVII) as P^1-8-hydroxy-7-methylisoalloxazin-10-ylribityl P^2-adenin-5'-yl diphosphate has not been definitively established, since no proof has been obtained of the stereochemical configuration of the polyhydroxyalkyl chain in the N-10 position as a D-ribityl substituent [118].

In addition to NADH dehydrogenase [116], a second enzyme has been isolated from *Peptostreptococcus elsdenii* which oxidizes NADH and contains a new orange prosthetic group — an electron-transferring flavoprotein (ETF). However, only this flavoprotein [116, 121] oxidizes NADH while simultaneously reducing the butyrylcoenzyme-A dehydrogenase. It has recently been shown that the apoproteins of NADH dehydrogenase and of *P. elsdenii* ETF are identical [122]. As has been found, the ETF isolated in the pure state contains FAD (III), 6-hydroxy-FAD (VII), and 8-hydroxynor-FAD (XVII) [121]. These flavins have been separated and the 8-hydroxynor-FAD has been isolated in the pure state [118]. After its enzymatic cleavage with phosphodiesterase, 8-hydroxynor-FMN (XVI) was isolated, and the hydrolysis of this with 1 N HCl in the dark led to 8-hydroxynor-riboflavin (XV) [118]. The photolytic cleavage of these two 8-hydroxyflavins in solution also yielded 8-hydroxy-7-methylalloxazine, but no proof of the stereochemical configuration of the substituent at N-10 as a D-ribityl group was given [118].

A riboflavin analog — 8-hydroxynorriboflavin, 8-hydroxy-7-methyl-10-(D-ribit-1'-yl)isoalloxazine (XV) — which is a component of the 8-hydroxynor-FAD molecule was synthesized by Polyakova, Tul'chinskaya, Zapesochnaya, and Berezovskii [123] in 1972 from 8-aminonorriboflavin by its diazotization in sulfuric acid solution followed by hydrolytic splitting of the flavin diazonium salt even before its presence in natural sources had become known.

8-Hydroxynorriboflavin (XV) has been synthesized by a new method from 5-amino-o-cresol, D-ribose, and violuric acid [124]. Compound (XV) is characterized by an absorption spectrum with λ_{max} 441 nm in the long-wave region; it exists in this form at pH 3.5 and below. Under physiological conditions, pH 6-8, and also at higher pH values, this compound exists in the tautomeric benzoquinoid oxo form with λ_{max} 472 nm [124]. When a solution of 8-hydroxynorriboflavin is photo-activated with UV light, intense yellow-green fluorescene is observed which, depending on the tautomeric state of the substance, has λ_{fl} 503 nm (hydroxy form; pH 3) or 526 nm (oxo form; pH 7) [124].



XV XVI

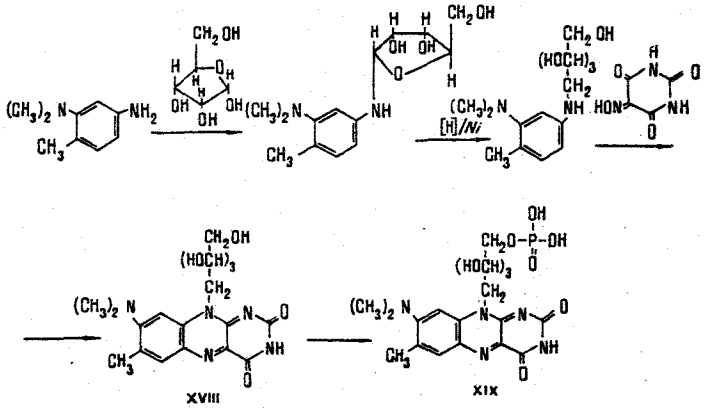
8-Hydroxynor-FMN (XVI) has been isolated from natural sources and its most important physicochemical properties have been determined [118]. 8-Hydroxynor-FMN, 1'-(8-hydroxy-7-methylisoalloxazin-10-yl)-D-ribit-5'-yl phosphate (XVI) has been obtained synthetically by Glebova and Berezovskii [124] from the flavin (XV) by phosphorylating its dimethyl ether with the acid chloride of orthophosphoric acid followed by isolation on ion-exchange resins. Like compound (XV), it exists in aqueous solutions in two tautomeric forms according to the pH and has similar absorption and fluorescence spectra.

8-Hydroxynor-FMN (XVI) and 8-hydroxynor-FAD (XVII) are apparently present as coenzymes

in the NADH dehydrogenases of animal tissues, since it is known that many vitamins and coenzymes are common both to animals and to microorganisms.

In recent years, new flavin growth factors or metabilites that are analogs of riboflavin have been discovered in certain microorganisms, but their biological role has not yet been established. Such flavins include: 8-dimethylaminorriboflavin (XVIII) from *Streptomyces davawensis*, 5'-formyldeoxymethylriboflavin (XXI) from *Schizophyllum commune*, and 5'-carboxydeoxymethylriboflavin (XXII) also from *Schizophyllum commune*.

8-Dimethylaminonorriboflavin (roseoflavin) (XVIII) was isolated in 1973 from *Streptomyces davawensis*, which was obtained from Phillipines soil [125]. The structure of the flavin (XVIII) was established in 1973-1974 by Miura, Matsui, Hirotsu, Shimoda, Takatsu, Otani, Nakano, and Kassi [125, 126] on the basis of the results of x-ray structural analysis [125] and was confirmed by the elementary analysis, the mass spectrum (M^+ m/e 574), and PMR spectrum of its acetyl derivative. The substituent in position 10 of the isoalloxazine ring was identified on the basis of a comparison of the physicochemical properties of the flavin (XVIII) and its tetraacetyl derivative with the properties of four synthesized diastereoisomers of 8-dimethylamino-7-methylisoalloxazin-10-yl-D-tetrahydroxypentane with the following tetrahydroxypentyl groups: D-ribityl, D-arabityl, D-xylityl, and D-lyxityl [126]. In addition, the PMR spectrum of the tetraacetyl derivative shows, from the chemical shifts of the $COCH_3$ protons and the similar intensities of the signals, that the peptide chain is not branched. The complete synthesis of roseoflavin (XVIII) was performed by Kasai, Miura, and Matsui [127] from 4-amino-2-dimethylaminotoluene, D-ribose, and violuric acid.



The absorption spectrum of the flavin (XVIII) is characterized by maxima (in water) at 225, 253, 314, and 505 nm [127]. The photolysis of compound (XVIII) under alkaline conditions leads to the partial demethylation of the tertiary amino group and to the formation of 8-monomethylaminonorriboflavin; in an acid medium the side chain is split off and 8-dimethylamino-7-methylalloxazine is obtained [127, 128]. 8-Dimethylaminonorriboflavin (XVIII) has a garnet-red color and, unlike riboflavin, does not possess fluorescence in aqueous solution; its ionization constants are pK_a 1.6 and 10.8, and its redox potential −0.466 V (relative to the SCE) [127]. This compound is reduced with considerably greater difficulty than riboflavin (−0.428 V). Compound (XVIII) possesses a weak antibiotic activity against Gram-positive bacteria [125, 126]. In tests for riboflavin activity on *Lactobacillus casei* and on mice, the flavin (XVIII) did not replace riboflavin, but showed a competitive inhibition effect [129]. The biosynthesis of the flavin (XVIII) in *S. davawensis* takes place from riboflavin through 8-amino- and 8-methylaminonorriboflavin [130].

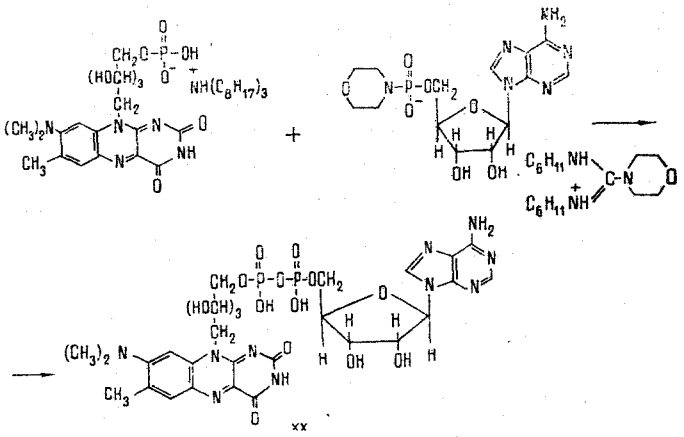
It must be mentioned that 8-aminonorriboflavin was synthesized for the first time in 1965, even before an amino analog of riboflavin with a tertiary amino group had been found in microorganisms, by the reaction of 4-methyl-6-phenylazo-N-D-ribityl-m-phenylenediamine with barbituric acid [115], in the form of orange-red needles possessing in dilute aqueous solutions the intense yellow-green fluorescence in UV light that is characteristic for many natural flavins. A secondary amine — 8-ribitylaminonorriboflavin was also obtained [115]. These two flavins proved to be the most powerful of the known competitive inhibitors of vitamin B_2 for a riboflavin-dependent strain of *Lactobacillus casei* [115], and they also exhibited a high competitive inhibitory activity (in a concentration of 6-12 μg/ml) in suppressing the growth of the strain *Bacillus subtilis* Rib B110 [132]. 8-Aminonorriboflavin [115] and 8-methylaminonorriboflavin [113] exhibited activity against coccidiosis of domestic poultry [134].

The condensation of substituted m-phenylenediamines with violuric acid has yielded several new riboflavin analogs with a dimethylamino group in position 8 [133]. Other 8-dialkylamino derivatives that have proved to be bacterial inhibitors have been synthesized [135].

An inhibiting influence of compound (XVIII) and of other dimethylamino analogs on the synthesis of 6,7-dimethyl-8-D-ribityllumazine (a biological precursor of riboflavin) and of riboflavin synthetase in strains of *B. subtilis* with different genetic states of the riboflavin operon has been found, the greatest antimetabolic activity being possessed by 8-dimethylaminorriboflavin (XVIII); the biological specificity of this flavin in relation to the biosynthesis of vitamin B_2 is confirmed by the isolation of mutants of *B. subtilis* resistant to this flavin and exhibiting the supersynthesis of riboflavin [133].

8-Dimethylaminonorriboflavin (XVIII) is possibly present in the molecule of the corresponding flavoproteins of the microbial cell in the form of its phosphorylated derivatives — analogs of FMN and FAD. Fetisova and Berezovskii [136] synthesized 8-dimethylaminorriboflavin mononucleotide (XIX) from the flavin (XVIII) by phosphorylation with dimethyl phosphoridomonochloridate followed by hydrolysis. Its absorption spectrum (in water) in the UV and visible region was similar to that of the flavin (XVIII). The pK_a values of 8-dimethylaminonor-FMN (XIX) are 4.8 and 8.8; the substances does not fluoresce in aqueous and alcoholic solutions. The biosynthesis of 8-dimethylaminonor-FMN has also been performed by the incubation of the flavin (XVIII) with rat liver flavokinase in the presence of ATP and Zn^{2+} [137].

In 1979, Litvak and Berezovskii [138] synthesized 8-dimethylaminonor-FAD (XX) from the tri-n-octylammonium salt of 8-dimethylaminonor-FMN and the N,N'-dicyclohexylmorpholine-4-carboxyamidinium salt of AMP.



The substance is characterized by an absorption spectrum (in water) with λ_{max} 259, 315, and 511 nm. This compound does not fluoresce in aqueous solution. On photo-activation by scattered light, the splitting out of one N-methyl group takes place with the formation of 8-monomethylaminonor-FAD, for which intense yellow-green fluorescence in water in UV light similar to the fluorescence of FAD, is characteristic [138].

In 1975, Tachibana, Murakami, and Ninomiya [139, 140] isolated from *Schizophyllum commune* and identified schizoflavins, the products of the partial or complete oxidation of the primary 5'-hydroxymethyl group of riboflavin: 5'-formyldoxymethylriboflavin (XXI) and 5'-carboxydenoxymethylriboflavin (XXII). The fact that *Sch. commune* contains new flavins had been reported previously [141, 142]. The absorption spectra of the compounds isolated were similar to the absorption spectrum of riboflavin. The photolysis of compounds (XXI) and (XXII) in an alkaline medium led to lumiflavin and in an acid medium to lumichrome, i.e., to the same compounds as are formed in the photolysis of riboflavin. Consequently, compounds (XXI) and (XXII) differed from riboflavin only by the side chain in position 10 [139]. The flavin (XXI) contains an aldehyde group, which was determined with the aid of the test for the catalytic acceleration of the oxidation of p-phenylenediamine by hydrogen peroxide. A carboxy group was detected in the flavin (XXII) by the color reaction of the iron salt of the hydroxamic acid, while it lacked the CH_2OH group that can be demonstrated for riboflavin by the absorption in the IR spectrum at 1060 cm^{-1} [140]. These schizoflavins are of interest in view of the fact that they apparently participate in the accumulation of L-malate in *Sch.*

commune [141] and stimulate the fermentation of carbohydrate-containing media by *Candida lipolytica* [143].

According to preliminary results [34], in some animal and plant materials the covalently bound flavins (i.e., without riboflavin and its coenzymes) amount to the following percentages of the total amount of flavins: in meat 65%, in wheat 73%, in peas 55%, in potatoes 60% It follows from this that riboflavin, FMN, and FAD are present in food products in far smalle amounts than the new flavin compounds.

It is quite possible that (amino acid)-substituted and modified analogs of riboflavin are vitamins but this question requires careful study. Thus, as a rule, the new natural analogs of riboflavin form with orthophosphoric acid or adenosine 5'-diphosphate a prosthetic group that is present in the reaction center of many flavoproteins: glycolate oxidase, trimethylamine dehydrogenase, succinate dehydrogenase, D-6-hydroxynicotine oxidase, sarcosine dehydrogenase, thiamine dehydrogenase, β-cyclopiazonate oxidocyclase, L-gulonolactone, D-gulonolactone dehydrogenase, cholesterol oxidase, monoamine oxidase, and NADH dehydrogenase, and they are present in a large group of other flavin enzymes the prosthetic groups of which have not yet been accurately identified.

The scientific advances of the last few years include the enormous potential possibility of the investigation of the distribution in nature of flavins already discovered and the sear for natural sources of new flavin vitamins, coenzymes, and other flavin compounds and a furth deepening of the study of the chemistry of the flavins and their role in the vital activity of enzymes.

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