Flavin–Pyridinium Biscoenzyme Analogs. Synthesis and Reactivity*

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ABSTRACT: The potential importance of flavin–pyridinium interactions in enzymic reactions catalyzed by flavoproteins has prompted us to undertake the synthesis of substances, embodying both a flavin and pyridinium group separated by a varying number of atoms, for study as model enzymes. The synthesis of flavin 3-carbamidopyridinium models with connecting links of 14, 9, and 7 atoms between the terminal flavin and pyridinium moieties F1-14-Nic (Ia), F1-9-Nic (Ib), and F1-7-Nic (Ic) as well as the flavin–pyridinium analogs F1-14-Pyr (Id) and F1-9-Pyr (Ie) is reported. The kinetics of the nonenzymatic oxidation of reduced nicotinamide–adenine dinucleotide (NADH) and dihydrolipoic acid ($Lip(SH)_2$) by these substances were determined. In all cases, rate enhancements of two- to sevenfold relative to riboflavin were observed. The results provide kinetic evidence for different mechanisms of participation by the pyridinium rings of the models in the oxidation of NADH and $Lip(SH)_2$. In the latter case, the rate increases which parallel closer proximity of the terminal flavin and pyridinium groups apparently reflect enhanced electronegativity of the flavin ring. However, in the oxidation of NADH, the relative rates, representative activation parameters, and control experiments indicate that the pyridinium ring actively participates in the transition state of the reductive process by coordination with the flavin ring or with NADH.

Riboflavin and substituted flavin derivatives have been used as models for flavoprotein activity, *e.g.*, the oxidation of $Lip(SH)_2$[1] (Gascoigne and Radda, 1967), reduced pyridine nucleotides (Singer and Kearney, 1950; Gascoigne and Radda, 1967), and 1-alkyl-1,4-dihydropyridine derivatives (Suelter and Metzler, 1960). In these model reactions an electron paramagnetic resonance signal has been observed and attributed to the flavin semiquinone. Careful studies have indicated that the semiquinone is formed as a result of disproportionation between completely reduced and oxidized flavin, rather than as a true reaction intermediate (Radda and Calvin, 1964; Gascoigne and Radda, 1967). In fact, all of the available evidence is consistent with a rate-limiting transfer of hydride ion from the donor to the flavin nucleus (Gascoigne and Radda, 1967; Radda and Calvin, 1964; Suelter and Metzler, 1960).

Flavinoid semiquinones have also been observed in analogous enzymic reactions (Massey *et al.*, 1961; Massey, 1963; Beinert and Sands, 1961). However, in the case of lipoyl dehydrogenase, which catalyzes the reversible reaction, NAD + reduced lipoic acids $\rightleftarrows$ NADH + oxidized lipoic acids + H^+, kinetic evidence has been presented that a red substance, presumably lipoyl dehydrogenase semiquinone, is an obligatory intermediate in both the forward and reverse directions (Massey *et al.*, 1961; Massey, 1963).

Since flavoprotein activity in these and other systems is intimately associated with pyridine nucleotides, we have undertaken the synthesis of substances which embody both a flavin and pyridinium ring with the anticipation that a study of the chemical and physical properties of the synthetic compounds may result in further elucidation of the mechanism of flavoprotein activity. In addition, there is the possibility that certain flavoproteins may function as binding surfaces for interaction of substrate with two coenzymes. Biscoenzymes, *i.e.*, two covalently linked coenzymes, and possibly biscoenzyme analogs may, therefore, be suitable models for particular enzymic activity.

Synthesis[2]

The flavin–carbamidopyridinium biscoenzyme analogs Ia (F1-14-Nic), Ib (F1-9-Nic), and Ic (F1-7-Nic) were prepared by melting together nicotinamide and the corresponding flavin ω-bromo esters (replace 3-carbamidopyridinium bromide by bromine in structure I) IIa (F1-14-Br), IIb (F1-9-Br), and IIc (F1-7-Br). The flavin–pyridinium analogs Id (F1-14-Pyr) and Ie (F1-9-Pyr) were obtained, similarly, from the corresponding flavin ω-bromo esters IIa and b after reaction with

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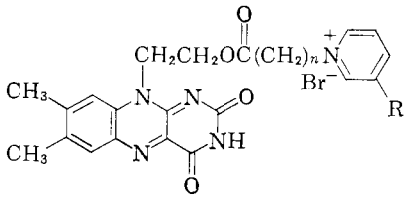
[1] Abbreviations used: $Lip(SH)_2$, dihydrolipoic acid; NADH, reduced nicotinamide–adenine dinucleotide; NAD, nicotinamide–adenine dinucleotide; NPrNH, 1-propyl-1,4-dihydronicotinamide; FAD, flavin–adenine dinucleotide.

[2] See Experimental Section for further details.

pyridine. The flavin ω-bromo esters II were formed by



esterification of the appropriate ω-bromo acid chloride with the known alcohol 7,8-dimethyl-10-[β-hydroxyethyl]isoalloxazine.

The biscoenzyme analogs were readily soluble in water in contrast to the corresponding bromo esters, and crystallized from water–acetone as well as methanol–ether solutions. Confirmatory evidence for the structures herein proposed was derived from ultraviolet spectral data which appear in Table I. Infrared spectra

TABLE I: Comparison of Relevant Ultraviolet Spectra.

λ_{max}^{MeOH} (mμ)	442	352	269	223
	$\epsilon \times 10^{-4}$			
Riboflavin	1.1	0.80	3.1	2.9
F1-14-Br (IIa)	1.2	0.81	3.2	3.2
F1-9-Br (IIb)	1.2	0.81	3.2	3.2
F1-7-Br (IIc)	1.2	0.81	3.2	3.1
F1-14-Nic (Ia)	1.2	0.80	3.5	3.8
F1-9-Nic (Ib)	1.2	0.83	3.6	3.8
F1-7-Nic (Ic)	1.2	0.82	3.6	3.8
F1-14-Pyr (Id)	1.2	0.83	3.5	3.4
F1-9-Pyr (Ie)	1.2	0.84	3.5	3.5
	ϵ/ϵ at 352 mμ			
Riboflavin	1.4	1.0	3.9	3.7
F1-14-Br (IIa)	1.5	1.0	3.9	4.0
Mixture[a]	1.4	1.0	3.9	3.9
F1-14-Nic (Ia)	1.5	1.0	4.5	4.9
Mixture[b]	1.5	1.0	4.6	4.8
F1-14-Pyr (Id)	1.5	1.0	4.2	4.2
Mixture[c]	1.5	1.0	4.2	4.0

[a] Equimolar mixture of riboflavin and methyl 11-bromoundecanoate. [b] Equimolar mixture of IIa and *N*-methyl-3-carbamidopyridinium chloride. [c] Equimolar mixture of IIa and *N*-ethylpyridinium bromide.

of the flavin ω-bromo esters and biscoenzyme analogs exhibit ester carbonyl absorption at *ca.* 5.7 μ (sh).

Results

In attempting to obtain evidence for interaction of the carbamidopyridinium group in reactions at the flavin site of the F1-Nic biscoenzyme analogs Ia–c,

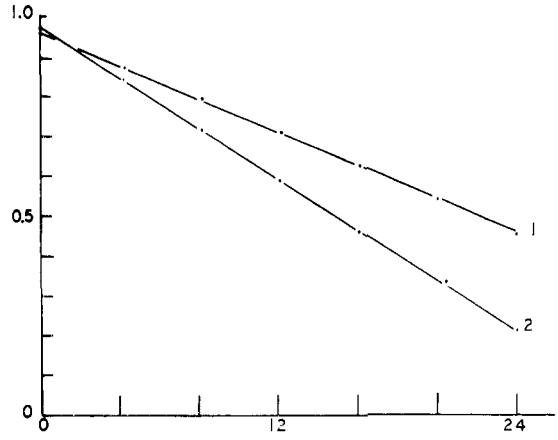


FIGURE 1. The rate of oxidation of NADH by F1-Nic biscoenzyme analogs. Abscissa: time in minutes; ordinate: $1 + (\log OD_t - OD_\infty)$ at 340 mμ; pH 7.8 (phosphate buffer, 0.1 ionic strength), 25°, and 1.84×10^{-4} M NADH. Curves 1 and 2: 2.4×10^{-4} M F1-14-Nic and F1-9-Nic, respectively.

the oxidation of NADH by the models was investigated. Since the analogs embody terminal flavin and pyridinium rings separated by seven to fourteen atoms, it was anticipated that the rate data might reflect an optimal chain length for maximal participation. As had been determined previously in the analogous oxidation of NPrNH by riboflavin (Suelter and Metzler, 1960), conducting the experiments aerobically resulted in clean *pseudo*-first-order oxidation of NADH owing to the rapid regeneration of the reduced flavins, which thereby functioned as catalysts. Typical first-order plots appear in Figure 1. Second-order rate constants, calculated from the expression $k_2 = k_1/[\text{flavin}]$, and rates relative to riboflavin appear in Table II. It is seen that the rate of oxidation of NADH by the F1-Nic analogs Ia–c is maximal for Ib, the model in which an intermediate number of atoms separates the terminal groups. Since the rate differences are small,[3] however, the related F1-Pyr analogs Id and e were prepared in order to determine the sensitivity of the kinetics to a structural change on the pyridinium ring. As noted in Table II, although the rate enhancements are comparable, there is a significant difference in the rate trends of the F1-Nic and F1-Pyr series.

In order to further assess the importance of intramolecular attachment of the pyridinium ring to the flavin, the oxidation of NADH by riboflavin was conducted in the presence of 1, 10, and 15 molar equiv of *N*-methyl-3-carbamidopyridinium chloride and *N*-

[3] Duplication of the experiments and changes in initial concentrations, within the ranges cited, resulted in second-order rate constants which agree within ±4%, thereby ensuring that the rate differences, although small, are real. Since riboflavin oxidizes NADH three times faster than does FAD at pH 7.35 (Singer and Kearney, 1950), the rate enhancements are *ca.* three times greater relative to FAD.

TABLE II: Comparison of Rates of Oxidation of NADH and Lip(SH)$_2$ by Riboflavin and Biscoenzyme Analogs, pH 7.8 (phosphate buffer), 25°.

Flavin	NADH Oxidation[a], k_2[c] (1 mole^{-1} sec^{-1})	Rel Rates	Lip(SH)$_2$ Oxidation,[b] k_2[d] (1 mole^{-1} sec^{-1} × 10^2)	Rel Rates
Riboflavin	0.775	1.0	3.87	1.0
F1-14-Nic (Ia)	3.34[e]	4.3	7.05	1.8
F1-9-Nic (Ib)	5.02	6.5	11.3	2.9
F1-7-Nic (Ic)	3.04	3.9	17.0	4.4
F1-14-Pyr (Id)	5.60	7.2	7.21	1.9
F1-9-Pyr (Ie)	3.44	4.4	11.1	2.9

[a] Ionic strength, 0.1; 1.84 × 10^{-4} M NADH; 1.20–2.53 × 10^{-4} M Flavin. [b] 0.2 ionic strength; 1.54–1.64 × 10^{-2} M Lip(SH)$_2$; 0.988–1.17 × 10^{-4} M Flavin. [c] ±4%. [d] ±6%. [e] 1.84–5.02 × 10^{-4} M NADH.

ethylpyridinium bromide. In all cases, rate diminutions of 10–20% as compared to oxidation by riboflavin alone were observed. Nevertheless, the significance of the presence of the pyridinium ring in the models is weakened by the small rate enhancements relative to riboflavin. Consequently, it was of interest to compare the corresponding activation parameters. Arrhenius plots for the oxidation of NADH by riboflavin and F1-14-Pyr appear in Figure 2. The corresponding enthalpies and entropies of activation, calculated according to Bunnett (1961), appear in Table III. The data reveal significant opposing differences in the activation parameters which result in the relatively small rate difference. It is seen that the presence of the pyridinium ring effects a considerable lowering of activation enthalpy at the expense of a greater loss in activation entropy.

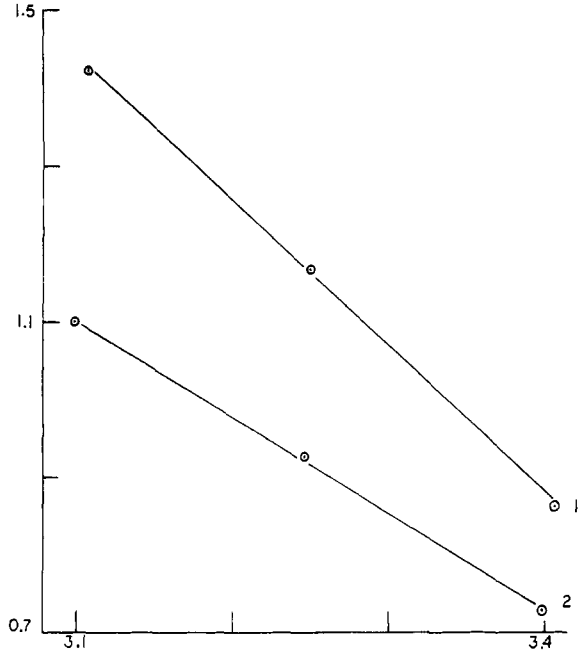


FIGURE 2: The rate of oxidation of NADH by riboflavin and F1-14-Pyr as a function of temperature. Each point represents an average of two runs. Curve 1: 0.968 × 10^{-4} M riboflavin and 2.44 × 10^{-4} M NADH; abscissa: $1/T \times 10^3$, ordinate $1 + \log k_2$ (second-order rate constant). Curve 2: 1.70 × 10^{-4} M F1-14-pyr and 2.49 × 10^{-4} M NADH; abscissa: $1/T \times 10^3$; ordinate: $\log k_2$.

In order to acquire evidence for the possible formation of an intermediate in these reactions, several attempts were made to observe saturation of F1-14-Nic, kinetically, by increased concentrations of NADH. When the concentration of F1-14-Nic was 2.0 × 10^{-4} M and that of NADH was varied from 1.2 to 5.0 × 10^{-4} M, initial rates were directly proportional to the initial concentration of NADH. Unfortunately, for NADH greater than 5 × 10^{-4} M, a gray solid rapidly precipitated from the solution thereby precluding spectrophotometric rate determinations.

While our work with NADH was in progress, the results of a study on the oxidation of Lip(SH)$_2$ by a series of flavin derivatives as a possible model system for lipoyl dehydrogenase were reported (Gascoigne and Radda, 1965). Since this flavoprotein catalyzes the reversible oxidation of Lip(SH)$_2$ by NAD (Massey *et al.*, 1961; Massey, 1963), it was of particular interest

TABLE III: Activation Parameters[a] for the Oxidation of NADH by Riboflavin and F1-14-Pyr at 25°, pH 7.8 (phosphate buffer), Ionic Strength 0.1.

Flavin	$\Delta H^{\ddagger}$ (kcal/mole)	$\Delta S^{\ddagger}$ (eu)
Riboflavin	8.0 ± 0.4	−34 ± 1
F1-14-Pyr	5.2 ± 0.4	−40 ± 1

[a] The limits were determined by least-square treatment of the rate data plotted in Figure 2.

to investigate the oxidation of $Lip(SH)_2$ by the biscoenzyme models, which embody an analog of NAD. Two aspects of the resulting rate data, which appear in Table II, are noteworthy. First, the rates are insensitive to a structural change on the pyridinium ring. Secondly, the rate enhancements appear to be directly related to the proximity of the positively charged pyridinium group and the isoalloxazine ring system. Both trends are contrasted in the NADH series.

Discussion

Since the initial report that flavin coenzymes catalyze the reduction of cytochrome *c* by reduced pyridinium nucleotides in the absence of cytochrome *c* reductase (Singer and Kearney, 1950), several groups have investigated the first stage of this process, reduction of flavin by reduced pyridine nucleotides, in order to determine whether the net two-electron change proceeds in one step by hydride transfer or by two one-electron transfers *via* the flavin semiquinone. Employing the NADH analog NPrNH, Suelter and Metzler (1960) determined that a hydrogen-transfer step is rate limiting. Later studies, utilizing electron paramagnetic resonance spectroscopy, indicated that a flavin semiquinone is formed but as a result of disproportionation between completely reduced and oxidized flavin, rather than as a true reaction intermediate (Radda and Calvin, 1964; Gascoigne and Radda, 1967). Both groups suggested that the rate-determining step is a direct transfer of hydride ion from the donor to the flavin nucleus, although, as pointed out by Suelter and Metzler, the evidence, including the electron paramagnetic resonance spectroscopy, does not exclude the slow transfer of a hydrogen atom occurring in a complex followed by a fast electron transfer. Evidence for formation of a weak complex between riboflavin 5′-phosphate and NADH at $-78°$ has been reported (Isenberg and Szent-Györgyi, 1959).

As a possible model system for the flavoprotein lipoyl dehydrogenase, which catalyzes the reversible oxidation of $Lip(SH)_2$ by NAD, Gascoigne and Radda (1967) studied the oxidation of $Lip(SH)_2$ as well as NADH by a series of substituted flavin derivatives. They found a direct correlation between the electronegativity of the substituted flavin, as measured by polarographic half-wave potentials, and the resulting rate of oxidation in both series. In analogy to the reaction of NADH with flavins, these results together with those of electron paramagnetic resonance spectroscopy were interpreted in support of a hydride-transfer mechanism in the $Lip(SH)_2$ series. In contrast to the model studies, however, kinetic evidence has been presented that a red substance, presumably lipoyl dehydrogenase semiquinone, is an obligatory intermediate in both the forward and reverse directions of the enzymic reaction (Massey *et al.*, 1961; Massey, 1963).

Perhaps the most significant experimental result of our studies is the observation of different trends in the rates of oxidation of NADH and $Lip(SH)_2$ by the biscoenzyme analogs. In the oxidation of $Lip(SH)_2$, the rate increases, which accompanies closer proximity of the terminal flavin and postively charged pyridinium groups, may be explained most simply by enhanced electronegativity of the flavin ring.[4] These results parallel the observations of Gascoigne and Radda (1967) on the oxidation of both $Lip(SH)_2$ and NADH by substituted flavin derivatives. However, the rate trends in the oxidation of NADH by the biscoenzyme analogs do not follow this simple pattern, indicative of a different mechanism of participation by the pyridinium ring of the models. Apparently, in contrast to simple flavin derivatives, the biscoenzyme models differentiate between the two hydrogen donors, thereby exhibiting a form of specificity.

In the oxidation of NADH by the biscoenzyme analogs: (1) the sensitivity of the kinetics to the number of atoms separating the terminal groups as well as to a structural change on the pyridinium ring, (2) the rate diminutions observed in the control experiments, and (3) the considerable lowering of activation enthalpy at the expense of a greater loss in activation entropy, all three factors strongly suggest that the pyridinium ring actively participates in the transition state of the reductive process by coordination with either the flavin ring or with NADH, possibly by enhancement of complexation between the reactants prior to reduction or by greater stabilization of the transition state in a one-step process. On the other hand, the significant differences in the activation parameters may possibly reflect a major difference in the mechanism of oxidation of NADH by riboflavin and the biscoenzyme models. Clearly, more definitive conclusions must rest on additional experimental data, particularly evidence for intermediates in these reactions. This work is in progress.

Further studies are necessarily required, as well, before any significance may be attached to the concept introduced herein of the possible utility of biscoenzyme analogs as suitable models for particular flavoprotein activity. In the interest of expanding the scope of this concept, we have initiated model enzyme studies with flavin–flavin biscoenzyme analogs, prompted by the large number of characterized flavoproteins which contain two flavins per mole of enzyme and the experimental data from optical and electron paramagnetic resonance studies which have been interpreted in terms of a possible intramolecular interaction between prosthetic flavins (Beinert and Sands, 1961).

Experimental Section[5]

Kinetics. Reaction rates were determined in a Cary

[4] Polarographic reduction of riboflavin and the biscoenzyme analogs did not reveal significant differences in the half-wave potential of the flavin ring, which is not inconsistent with this possibility in view of the small rate differences. We are indebted to Miss Ellen Coffey for these determinations.

[5] Melting points are corrected. Analyses were carried out by Spang, Ann Arbor, Mich., and Midwest Microlab, Indianapolis, Ind.

14 recording spectrophotometer which was equipped with a thermostated cell compartment. In a typical experiment in the NADH series, 2 ml of buffer solution containing the flavin derivative was pipetted into a silica cell of 1-cm path length, followed by 0.1 ml of an aqueous solution of NADH. The cuvet was shaken, and the optical density was recorded at 340 mμ against a blank containing all reactants except NADH. Fresh buffer solutions of flavin and aqueous solutions of the sodium salt of NADH were prepared daily for each series of experiments.

In the $Lip(SH)_2$ series, argon was passed through buffered solutions of both $Lip(SH)_2$, contained in the cuvet, and the flavin derivative *via* syringe needles. Both containers were stoppered with syringe caps which were fixed with a syringe needle for release of pressure. After 20 min, the required amount of flavin was transferred to the $Lip(SH)_2$ solution with a microsyringe; the pressure-release and argon-flow needles were removed from the cuvet in that order; and the optical density at 442 mμ was recorded. Plots of log $(OD_t - OD_\infty)$ *vs.* time afforded *pseudo*-first-order rate constants since the concentration of $Lip(SH)_2$ in these experiments was at least 100 times greater than that of flavin. Second-order rate constants were calculated from the expression $k_2 = k_1/[Lip(SH)_2]$.

Reduced Nicotinamide–Adenine Dinucleotide. The sodium salt of NADH, λ_{max}^{water} 340 mμ (ϵ 5.2 × 10^3), was purchased from Pabst Laboratories and used without further purification.

dl-Dihydro-α-lipoic acid was prepared from *dl*-α-lipoic acid (purchased from Farmochimica Cutolo-Calosi, Naples, Italy) as previously described (Wagner *et al.*, 1956).

7,8-Dimethyl-10-isoalloxazineethyl 6-Bromohexanoate) (IIb). A mixture of 7,8-dimethyl-10-[β-hydroxyethyl]isoalloxazine (Fall and Petering, 1956) (1.18 g) and 6-bromohexanoyl chloride (obtained from Eastman Organic Chemicals) (2.50) was stirred under a stream of nitrogen in refluxing acetonitrile (25 ml) for 48 hr and then overnight at room temperature. The resulting mixture was filtered to afford 1.17 g of solid material which was taken up in 70 ml of hot methanol. The methanol solution was cooled to room temperature, filtered, and the supernatant was concentrated to afford 929 mg of crude ester, an orange-yellow solid. The methanol-insoluble material was unreacted alcohol.

The acetonitrile supernatant from the initial filtration was concentrated to a residual oil which crystallized on trituration with ether. After being filtered and washed well with ether, this material was combined with the above ester for a total crude yield of 1.61 g (83%), mp (*in vacuo*) 210–212° dec. The melting point was 214–215° after two recrystallizations from methanol–ether.

Anal. Calcd for $C_{20}H_{23}BrN_4O_4$; C, 51.8; H, 5.0; N, 12.1. Found: C, 51.7; H, 4.7; N, 12.3.

The related esters IIa (mp (*in vacuo*) 202–203° dec) and IIc (mp (*in vacuo*) 206–207° dec) were obtained by the same procedure in similar yields from 11-bromoundecanoyl chloride (Blackman and Dewar, 1957) and 4-bromobutanoyl chloride (purchased from K & K Laboratories), respectively.

Anal. (for IIa) Calcd for $C_{25}H_{33}BrN_4O_4$: C, 56.3; H, 6.2; N, 10.5. Found: C, 56.4; H, 6.3; N, 10.7.

Anal. (for IIc) Calcd for $C_{18}H_{19}BrN_4O_4$: C, 49.7; H, 4.4; N, 12.9. Found: C, 50.7; H, 4.7; N, 13.3.

7,8-Dimethyl-10-isoalloxazineethyl-4-[1-(3-carbamido)pyridinium Bromide]butanoate (Ic). An intimate mixture of IIc (322 mg) and nicotinamide (2.1 g) in a 10-ml round-bottom flask was heated and stirred under nitrogen at 125–130° for 10–15 min. The resulting melt was taken up in methanol, transferred to a 250-ml round-bottom flask, and concentrated to a residual oil which solidified after several washings with acetone. The solid was taken up in a small amount of water (2–3 ml) and acetone was added to induce crystallization. The product (242 mg) was obtained after an additional crystallization from water–acetone or methanol–ether solutions. Analytically pure material (mp (*in vacuo*) 189–190° dec) was obtained after several recrystallizations from water–acetone.

Anal. Calcd for $C_{24}H_{25}BrN_6O_5$: C, 51.7; H, 4.5; N, 15.1. Found: C, 51.4; H, 4.8; N, 15.4.

The related biscoenzyme analogs Ia (mp (*in vacuo*) 216–219° dec) and Ib (mp (*in vacuo*) 224–226° dec) were prepared by the same procedure in similar yields from the corresponding flavin ω-bromo esters.

Anal. (for Ia) Calcd for $C_{31}H_{39}BrN_6O_5$: C, 56.8; H, 6.0; N, 12.8. Found: C, 57.1; H, 6.3; N, 12.8.

Anal. (for Ib) Calcd for $C_{26}H_{29}BrN_6O_5$: C, 53.3; H, 5.0; N, 14.4. Found: C, 53.6; H, 5.1; N, 14.3.

7,8-Dimethyl-10-isoalloxazineethyl 11-[1-Pyridinium Bromide]undecanoate (Id). A solution of IIa (245 mg) in pyridine (10 ml) was refluxed for 2 hr and, after standing at room temperature for 2 days, was filtered through sintered glass. The filtrate was evaporated to dryness and triturated with acetone to afford an orange solid (249 mg). Several recrystallizations from absolute ethanol afforded analytically pure material, mp (*in vacuo*) 147–148° dec.

Anal. Calcd for $C_{30}H_{38}BrN_5O_4$: C, 58.8; H, 6.3; N, 11.4. Found: C, 58.6; H, 6.4; N, 11.4.

The related analog Ie (mp (*in vacuo*) 165–166° dec) was prepared by the same procedure from IIb.

Anal. Calcd for $C_{25}H_{28}BrN_5O_4$: C, 55.4; H, 5.2; N, 12.9. Found: C, 55.4; H, 5.2; N, 12.9.

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Sterol Metabolism. I. 26-Hydroxycholesterol in the Human Aorta*

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ABSTRACT: 26-Hydroxycholesterol has been isolated from healthy and diseased human aortal tissue by means of column, thin layer, and gas chromatographic procedures and identified by comparison of the sterol and its 3β,26-diacetate with authentic samples. Twenty odd sterol-like components present in the polar lipid fraction from the human aorta have been resolved, and 7-ketocholesterol, 25-hydroxycholesterol, 7α-hydroxycholesterol, 7β-hydroxycholesterol, and 5α-cholestane-3β,5,6β-triol have been recognized among the components. 26-Hydroxycholesterol appears to be confined to intimal tissue alone. Gas chromatographic analyses indicate that 26-hydroxycholesterol is present in the human aorta at levels of *ca.* 32 μg/g of dry intimal tissue or 4–100 μg/g of dry combined intimal and medial tissue.

The artifact nature of the 7-hydroxycholesterols and of 25-hydroxycholesterol is suggested, and the role of 26-hydroxycholesterol as a cholesterol companion sterol in the human aorta is considered.

The presence of low levels of other sterols in mammalian tissue cholesterol[1] samples is well recognized. Chief among these minor sterols are cholestanol, 5α-cholest-7-en-3β-ol, cholesta-5,7-dien-3β-ol, cholest-5-ene-3β,24*S*-diol, 7-ketocholesterol, and 5α-cholestane-3β,5,6β-triol. In addition other cholesterol biosynthesis intermediates may be encountered in select cases. Other than sterol biosynthesis intermediates the biological role of the companion sterols is obscure, even for the predominant cholestanol, which has been recognized as a cholesterol companion for 40 years.

Among the tissues most studied for minor sterol composition is the human aorta, with its obvious relation to atherosclerosis. The presence of cholesterol in the diseased human aorta was recognized early (Vogel, 1847). By the early 20th century both cholesterol (Aschoff, 1906; Windaus, 1910; Schoenheimer, 1926, 1928) and cholestanol (Schoenheimer *et al.*, 1930; McArthur, 1942; Mosbach *et al.*, 1963; Kuroda *et al.*, 1964) were established as component sterols in the human aorta.

The levels of cholesteryl fatty acid esters in the human aorta have been extensively reviewed (Tuna and Mangold, 1963). Furthermore, the presence of desmosterol in aortal tissue of patients treated with triparanol has been reported (Blankenhorn *et al.*, 1961; Chobanian and Hollander, 1961, 1965; Jose and Peak, 1963). Other sterols isolated from or detected in the human aorta include cholesta-3,5-dien-7-

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[1] Systemic nomenclature for steroids given trivial names in the text includes: cholest-5-en-3β-ol, cholesterol; cholest-5-ene-3β,26-diol, 26-hydroxycholesterol; 5α-cholestan-3β-ol, cholestanol; cholesta-5,24-dien-3β-ol, desmosterol; cholest-5-ene-3β,7α-diol, 7α-hydroxycholesterol; cholest-5-ene-3β,7β-diol, 7β-hydroxycholesterol; 3β-hydroxycholest-5-en-7-one, 7-ketocholesterol; cholest-5-ene-3β,24*S*-diol, 24-hydroxycholesterol (cerebrosterol); cholest-5-ene-3β,25-diol, 25-hydroxycholesterol. The 7β-hydroxycholesterol nomenclature used by Hardegger *et al.* (1943), Henderson and MacDougall (1954), and Henderson (1956) appears to refer to the 7α-epimer and has been so altered herein to conform with the correct configurational assignments for these sterols.