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Alkylation of Flavins Using Methyl Fluorosulfonate[a]

Short Communication

Peter C. Preusch[b] and **Donald B. McCormick***, [c]

Section of Biochemistry, Molecular and Cell Biology and
Division of Nutritional Sciences, Cornell University, Ithaca, NY 14853, U.S.A.

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A method for N^3 methylation of flavins is described using methyl fluorosulfonate. The reaction proceeds through O alkylation of the carbonyls followed by an O to N rearrangement.

(Keywords: Alkylation; Flavin; Methyl fluorosulfonate; Rearrangement, N—O)

Über die Alkylierung von Flavinen mit Fluorsulfonsäuremethylester (Kurze Mitteilung)

Es wird eine Methode zur N^3-Methylierung von Flavinen mittels Fluorsulfonsäuremethylester beschrieben. Die Reaktion verläuft über eine O-Methylierung der Carbonylgruppe mit anschließender Methylwanderung vom Sauerstoff an den Stickstoff.

N^3 alkylation of flavins has been used to enhance solubility, append additional molecular groupings, and to simplify kinetic analyses by eliminating contributions to reaction *pH* dependence due to ionization at this position. 3-Methylflavins have been particularly widely employed. These may be prepared by condensation of suitable precursors to form the isoalloxazine nucleus[1,2]. This approach, however, requires many steps to prepare the immediate precursors and may not be

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[b] Present address: Department of Biochemistry, University of Wisconsin, 420 Henry Mall, Madison, WI 14853, U.S.A.

[c] Present address: Department of Biochemistry, Emory University School of Medicine, Woodruff Memorial Building, Atlanta, GA 30322, U.S.A.

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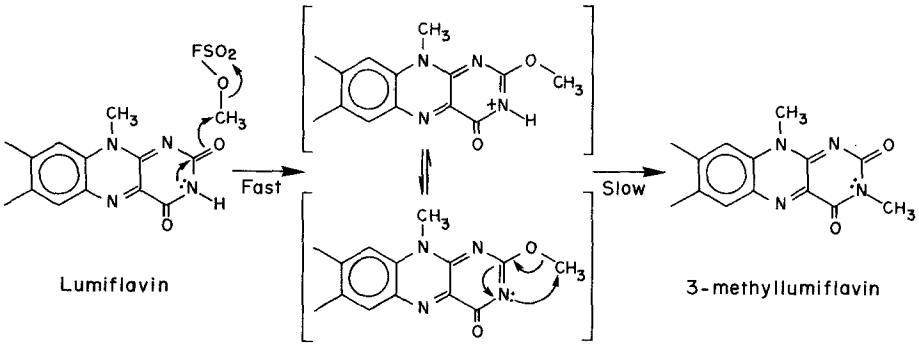
suitable in certain applications. Alkylation of the intact flavin using dimethyl sulfate[3] or methyl iodide[4,5] in anhydrous media in the presence of potassium carbonate[3,4] or silver oxide[5] has been more frequently employed. These methods suffer from the occurrence of base-catalyzed side reactions, notably biflavin formation[6]. Alkylation with methyl fluorosulfonate in the inert solvent dimethyl acetamide[7] has been found to avoid these side reactions.



All manipulations were carried out at room temperature in dim light. The course of the reaction was followed by thin-layer chromatography on precoated sheets of silica gel N-HR (Brinkman, Westbury, N.Y., U.S.A.) developed in benzene: glacial acetic acid: water (11:11:2, upper phase). Compounds were detected by their appearance under visible and ultraviolet light.

Lumiflavin (**1**) (0.054 g, 0.208 mmol) was dissolved in 80 ml of dry dimethyl acetamide (Aldrich, Milwaukee, Wi., U.S.A.). Methyl fluorosulfonate (8 ml) (Aldrich) was added in one-ml aliquots over a period of 4 h with stirring, then the reaction was left to proceed overnight in a stoppered flask. No 3-methyllumiflavin ($R_f = 0.35$) was formed; but rather, a weakly fluorescent, unknown yellow material (X) appeared at the origin. Ultimately, about 80% of the initial starting material ($R_f = 0.22$) was converted to X. This material along with unreacted lumiflavin was extracted into chloroform (three 50-ml portions) after addition of 100 ml of potassium phosphate buffer (0.1 M, pH 7.4). The chloroform extracts were washed with four 100-ml portions of water which were in turn back-washed with the same 25-ml portion of chloroform. The pooled chloroform layers were dried over anhydrous sodium sulfate, filtered, and the filtrate concentrated *in vacuo* at 38 °C to about 20 ml. The concentrated extracts were dropped into 200 ml of

ice-cold diethyl ether. The precipitate that formed on standing overnight at —20 °C was collected by filtration.

Analysis of the precipitate and filtrate fractions showed the presence of 3-methyllumiflavin (*cf.* 80% of the total material) and unreacted lumiflavin; no compound *X* was present. The filtrate which contained most of the desired material was evaporated to dryness *in vacuo* at 30 °C. The residue was redissolved in a minimum volume of chloroform and fractionally precipitated with petroleum ether (b.p. 30-60 °C, Mallinkrodt, St. Louis, Mo., U.S.A.) to give 20 mg (0.074) mmol of 3-methyllumiflavin for a 35% yield.

The material so obtained was found to be identical with authentic 3-methyllumiflavin[5,6] by thin-layer chromatography, absorption, and nuclear magnetic resonance analyses.

The behavior of compound *X* suggests formation of a positively charged species that is deprotonated at *pH* 7.4. By analogy to other known reactions of methyl fluorosulfonate[8], it may be proposed that the reaction proceeds through O methylation of the carbonyl functions with quaternization of the N^3 position yielding the intermediate *X* as shown in the reaction below*. On standing an O to N rearrangement must occur yielding the N^3-methylated product. The proposed iminol ester (iminoether) intermediate has been prepared by other means[9,10]; its properties, notably $pKa = 3.7$, are consistent with the observations reported here. None of the known multiply-alkylated flavin structures[11] are consistent with them.

Methylation using methyl fluorosulfonate offers some advantages over the previous alkylation procedures. No side-reactions occur; unconverted starting material may be cleanly recovered. The overall yield of desired product is comparable to other methods; the yield in the actual reaction is higher. An improvement in overall yield might be obtained by revising the work-up to take advantage of the very different properties of the intermediate *X* from those of lumiflavin. The reaction should be applicable to other flavins and also other alkyl groups, limited only by the availability of suitable fluorosulfonate reagents.

References

[1] *P. Hemmerich, S. Fallab,* and *H. Erlenmeyer,* Helv. Chim. Acta **39**, 1242 (1956).

[2] *P. Lamboy,* in: Methods in Enzymology XVIII, p. 437, (*D. B. McCormick* and *L. W. Wright,* eds.). New York: Academic Press. 1971.

* Alkylation at O^2 is shown. Similar properties would be expected of O^4-alkylated material; both may occur.

[3] *P. Hemmerich, B. Prijs*, and *H. Erlenmeyer*, Helv. Chim. Acta **43**, 372 (1960).
[4] *P. Hemmerich*, Helv. Chim. Acta **47**, 464 (1964).
[5] *P. Brown, C. L. Hornbeck*, and *J. R. Cronin*, Org. Mass Spec. **6**, 383 (1972).
[6] *P. Hemmerich, B. Prijs*, and *H. Erlenmeyer*, Helv. Chim. Acta **42**, 2164 (1959).
[7] *M. G. Ahmed, R. W. Adler, G. H. James, M. L. Sinnot*, and *M. C. Whiting*, Chem. Commun. **1968**, 1533.
[8] *M. G. Ahmed* and *R. W. Adler*, Chem. Commun. **1969**, 1389.
[9] *F. Müller, W. Walker*, and *P. Hemmerich*, Helv. Chim. Acta **49**, 2365 (1966).
[10] *K. H. Dudley* and *P. Hemmerich*, Helv. Chim. Acta **50**, 355 (1967).
[11] *K. H. Dudley, A. Ehrenberg, P. Hemmerich*, and *F. Müller*, Helv. Chim. Acta **47**, 1354 (1964).