0.70 ml of 1.66 *M* *n*-butyllithium in hexane under nitrogen. The reaction mixture was heated at *ca.* 65° for 7 hr and then left for 10 hr at room temperature. After removal of methanol under reduced pressure, the product was isolated by addition of water and extraction with ether. The combined ethereal extracts were washed with 2% sodium hydroxide solution and saturated sodium chloride solution and dried over magnesium sulfate. Removal of the solvents *in vacuo* produced the crude product. Purification by preparative tlc on silica gel plates using ether–hexane (2:1) afforded 127 mg (58%) of racemic (*E*)-nuciferol which was homogeneous by glc (SE-30): ir (film) 3340 cm^{-1}; nmr (CCl_4) δ 1.21 (d, 3 H), 1.50 (s, 3 H), 1.60–2.15 (m, 5 H), 2.30 (s, 3 H), 2.60 (m, 1 H), 3.82 (s, 2 H), 5.28 (b t, 1 H), 6.98 (s, 4 H); mass spectrum *m/e* 218.

Anal. Calcd for $C_{15}H_{22}O$: C, 82.51; H, 10.16. Found: C, 82.73; H, 10.15.

Registry No.—**1**, 39599-18-3; **2a**, 39533-45-4; **2b**, 39533-46-5; **2c**, 39533-47-6; **3** (R = H), 37616-05-0; **5** (R = H), 513-42-8; β-methyl-4-methylcinnamic acid, 14271-34-2; *p*-toluenesulfenyl chloride, 933-00-6.

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Isotopic Labeling Studies of the Base-Catalyzed Conversion of 1-Methyladenosine to N^6-Methyladenosine

MARY H. WILSON AND JAMES A. MCCLOSKEY*

Institute for Lipid Research and Department of Biochemistry, Baylor College of Medicine, Houston, Texas 77025

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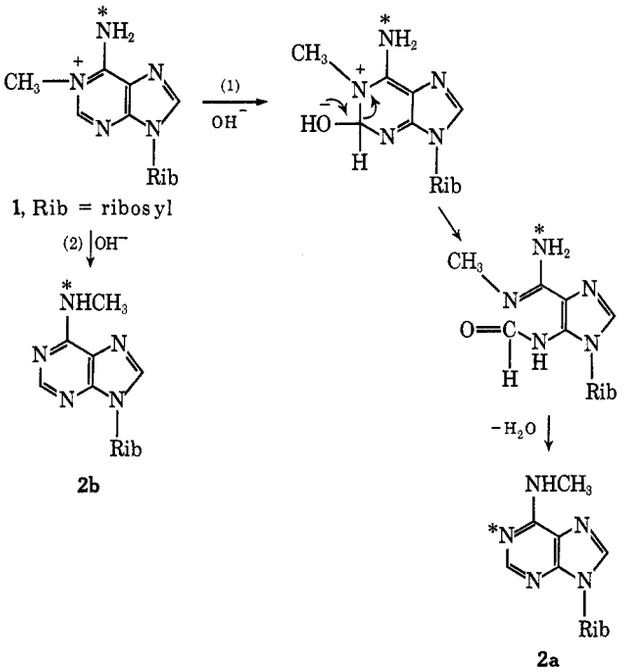
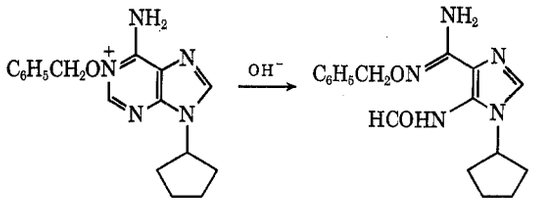
The observation that certain derivatives of 1-methylpurine rearrange in base to N^6-methyladenine was reported over a decade ago,[1,2] and has since been shown to occur for a variety of purine derivatives and has often been the basis for synthesis of N^6-substituted derivatives of adenine.[3] Following the proposal of Taylor and Loeffler, who studied the structurally similar pyrazolo[3,4-*d*]pyrimidine system,[4] the mechanism has been generally presumed to follow that of the Dimroth rearrangement,[5] involving ring opening and recyclization (eq 1) rather than simple methyl migration (eq 2).

In related work, Windmueller and Kaplan studied the ring opening of 1,6-bis(2-hydroxyethylamino)-9-(β-D-ribofuranosyl)purine in base.[6] An intermediate ring-opened product, which does not undergo recyclization



in dilute alkali, was isolated as a diazo derivative, but did not have a structure analogous to the intermediate shown in eq 1. The most detailed study to date is that of Macon and Wolfenden, who failed to detect or trap an intermediate species in the conversion of **1** to **2** but found that the reaction occurs at room temperature from ~pH 7 to 13 and follows pseudo-first-order kinetics.[7] Their data were interpreted in terms of an initial ring opening (eq 1) brought about by attack of hydroxide on the neutral or protonated form of 1-methyladenosine. In more recent work, Montgomery and Thomas have isolated the intermediate formamide derivative **4** derived from 1-benzyloxy-9-cyclopentyladenine (**3**), which was then converted with ring closure



to the N^6-benzyloxy derivative.[8] From these and other less relevant data[9,10] it has been reasonably assumed[7–10] that rearrangement of 1-substituted purines in base follows the Dimroth mechanism, in analogy to the pyrimidines, for which the overall mechanism has been clearly established.[5,11–13]

The present study of the conversion of 1-methyl-6-amino-9-(β-D-ribofuranosyl)purine (**1**) to 6-methylamino-9-(β-D-ribofuranosyl)purine (**2**) was undertaken to directly test the mechanism in eq 1 by use of ^{15}N and

(1) (a) A. R. Todd in "CIBA Foundation Symposium on the Chemistry and Biology of Purines," G. E. W. Wolstenholme and C. M. O'Connor, Ed., Little, Brown and Co., Boston, Mass., 1957, p 57; *cf.* G. B. Elion, ref 1a, p 44.

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(3) For example, (a) A. Codington, *Biochem. Biophys. Acta*, **59**, 472 (1962); (b) G. B. Elion, *J. Org. Chem.*, **27**, 2478 (1962); (c) J. W. Jones and R. K. Robins, *J. Amer. Chem. Soc.*, **85**, 193 (1963); (d) P. D. Lawley and P. Brookes, *Biochem. J.*, **89**, 127 (1963); (e) W. Grimm, T. Fuji, and N. J. Leonard in "Synthetic Procedures in Nucleic Acid Chemistry," Vol. 1, W. W. Zorbach and R. S. Tipson, Ed., Wiley-Interscience, New York, N. Y., 1968, p 212; (f) H. T. Nagasawa, P. S. Fraser, and J. A. Elberling, *J. Org. Chem.*, **37**, 516 (1972).

(4) E. C. Taylor and P. K. Loeffler, *J. Amer. Chem. Soc.*, **82**, 3147 (1960).

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(13) J. Goerdeler and W. Roth, *Chem. Ber.*, **96**, 534 (1963).

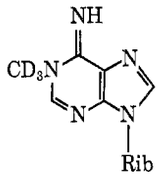
TABLE I

RELATIVE ION ABUNDANCE DATA FROM THE MASS SPECTRA OF 2 AND PRODUCTS OF CONVERSION OF ISOTOPICALLY LABELED 1

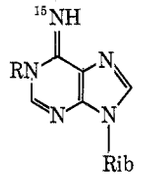
	m/e				
Starting compd	120	121	122	123	124
1 (pH 11)	100	62.6 ± 2.0	7.7 ± 0.6		
5 (pH 11)	37.3 ± 0.2	100	46.2 ± 0.5	32.4 ± 0.3	
6[a] (pH 11)	22.2 ± 0.8	100	59.6 ± 1.0	7.7 ± 0.3	
7[a] (pH 7)	11.4 ± 1.8	44.6 ± 1.1	100	47.2 ± 0.8	31.0 ± 0.6
7[a] (pH 11)	15.3 ± 0.9	47.9 ± 0.7	100	46.9 ± 0.6	29.6 ± 0.4

[a] Abundance data corrected for presence of 9.6% ^{14}N.

D labeling, and in addition to demonstrate the utility of mass spectrometry for dealing with mechanistic problems of this type. Mass spectrometry of the reaction products, without the usual degradation to volatile gases, provides a rapid and sensitive means of determining the location and extent of isotopic labeling. The conversion of **1** to **2** was studied both at neutrality and pH 11, using compound **1** specifically labeled with ^{15}N (**5**). Mass spectra of N^6-methyladenosine and its analogs[14] have been studied in detail, permit unambiguous differentiation of the products resulting from isomerization and methyl migration (**2a** *vs.* **2b**), and can be used as a quantitative test for the existence of competing pathways down to a level of ~2% at both pH values. The deuterium-labeled analogs **6** and **7**



6



5, R = CH_3

7, R = CD_3

were also examined under the same conditions in order to unambiguously confirm the interpretation of the mass spectra.

Detailed understanding of the mechanism of this reaction is important, not only because of its role in synthetic procedures (*e.g.*, ref 3a,e,f) but also because of the possibility of its occurrence during isolation and chemical treatment of tRNA or oligonucleotides which contain 1-methyladenine residues.[15] The reaction is also potentially useful for the preparation of isotopically labeled analogs of N^6-substituted adenosine for chemical or biological studies.

Experimental Section

Mass spectra were recorded on an LKB 9000 instrument with sample introduction by direct probe: ion source temperature 250°, ionizing electron energy 70 eV. The ion abundance measurements reported represent the mean of at least six consecutively recorded partial spectra.

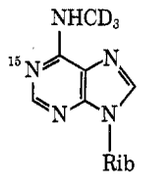
Adenosine-$^{15}N^6$[16]—6-Chloro-9-(β-D-ribofuranosyl)purine (100 mg) and methanol (1 ml) were placed in a 3-ml reaction vessel and evacuated to 3 mm pressure. $^{15}NH_3$ (0.5 l., Bio-Rad Laboratories, 90% ^{15}N) was distilled into the cooled vessel, which was then sealed and allowed to stand for 16 hr at room temperature and then 3 hr at 40°. Evaporation of excess $^{15}NH_3$ and methanol yielded 90 mg of chromatographically pure adenosine-$^{15}N^6$.

1-Methyladenosine (**1**), **1-methyladenosine-$^{15}N^6$** (**5**), **1-(methyl-d_3)adenosine** (**6**), **and 1-(methyl-d_3)adenosine-$^{15}N^6$** (**7**) were prepared as hydriodide salts from adenosine or adenosine-$^{15}N^6$ and CH_3I or CD_3I (Merck Sharp and Dohme of Canada, 99% D) in *N*,*N*-dimethylacetamide by the method of Jones and Robins.[3c] The above five products exhibited the expected mass spectra[14] with suitable mass shifts due to presence of ^{15}N or D, and which showed no contamination from starting material or other products.

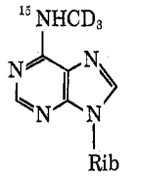
Treatment with Base and Purification of Products.—1-Methyladenosine hydriodide (**1**) or its isotopically labeled analogs (1 mg) were added to water (0.5 ml) and the pH was adjusted to 7 or 11 with 0.25 *M* NaOH. The solution was heated at 100° for 2 hr and cooled. The pH was adjusted to 5.0 with 1 *N* HCl and the mixture was applied to a 5 × 60 cm column of Dowex 50-X8. N^6-Methyladenosine (**2**) or its labeled analogs were eluted with 0.4 *M* ammonium formate, pH 5.0. The product obtained after lyophilization and removal of buffer salt under vacuum was chromatographically and mass spectrometrically pure.

Results and Discussion

If conversion of **1** to **2** proceeds exclusively by Dimroth rearrangement (eq 1), the methyl group at N-1 should appear at N^6 in the product, and the identity of N-1 and N^6 nitrogens should be reversed. The doubly labeled compound **7** would therefore yield **8**, while contributions from direct methyl migration (eq 2) would yield proportional amounts of the isotopic isomer **9**. Location of the isotopic labels in



8



9

the rearranged products from **5**, **6**, and **7** can be established mass spectrometrically using the peak representing loss of methyleneimine from the base + H fragment ion, a reaction characteristic of the N^6-methyladenine moiety.[14,17-19] Interchange of amino and methyl hydrogen occurs during decomposition of *m/e* 149,[19] resulting in a slightly more complex ion pattern in the *m/e* 120 region in the case of the deuterated models **6** and **7**. However, the location of the methyl group in relation to the nitrogen at N^6 in **1** can be clearly established by mass shifts of the *m/e* 120 ion-type derived from **5**, **6**, and **7**. Expulsion of CH_2N from *m/e* 149 produces a companion peak of *m/e* 121 which bears the same information (Scheme 1).

Ion abundance for the diagnostic *m/e* 120 region of the spectra are presented in Table I. From the pH 11 experiment, the isotopic pattern from **7** is identical with

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(16) T. Sato in "Synthetic Procedures in Nucleic Acid Chemistry," Vol. 1, W. Zorbach and R. Tipson, Ed., Wiley-Interscience, New York, N. Y., 1968, p 264.

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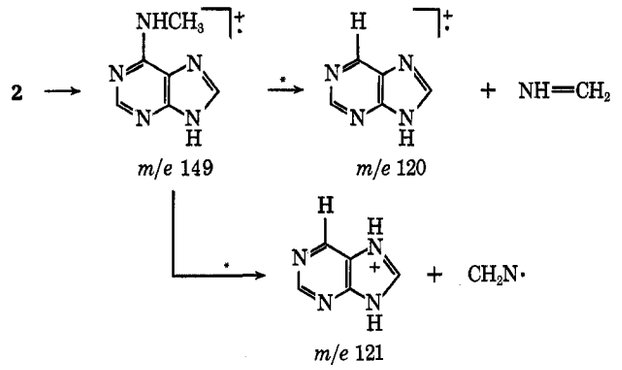
(19) D. L. von Minden, J. G. Liehr, and J. A. McCloskey, Twenty First Annual Conference on Mass Spectrometry and Allied Topics, San Francisco, Calif., May 1973.

SCHEME I



that of **5** but shifted one mass unit higher, showing the presence of ^{15}N originating at N^6 in **1**, in support of the ring-opening mechanism. This interpretation is confirmed by the singly labeled derivative **6**, which produces a pattern which is experimentally indistinguishable from **1** but offset one mass unit higher as required by the Dimroth mechanism. The absence of contributions from products such as **9** which would contain an exocyclic ^{15}N label shows the absence of a methyl migration mechanism (eq 2) at the detectable limit of approximately 2% of the total reaction yield.

Conversion of 1-methyladenosine was more than 90% complete at pH 11 under the conditions employed, but less than 50% complete at pH 7. However, the identity of patterns from compound **7** at the two pH values shows that transformation to N^6-methyladenosine occurs exclusively by ring opening in both the protonated (pH 7) and neutral (pH 11) forms of 1-methyladenosine, a result which is consistent with earlier kinetic studies of Macon and Wolfenden.[7]

Registry No.—**1**, 34308-25-3; **2**, 1867-73-8.

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Biosynthesis of Ergot Alkaloids. Synthesis of 6-Methyl-8-acetoxymethylene-9-ergolene and Its Incorporation into Ergotoxine by *Claviceps*

C.-C. LESLIE LIN, GARY E. BLAIR, JOHN M. CASSADY, DETLEF GRÖGER, WALTER MAIER, AND HEINZ G. FLOSS*

Department of Medicinal Chemistry and Pharmacognosy, Purdue University, West Lafayette, Indiana 47907, and the Institute for Biochemistry of Plants, Academy of Sciences of the GDR, Halle (Saale), German Democratic Republic

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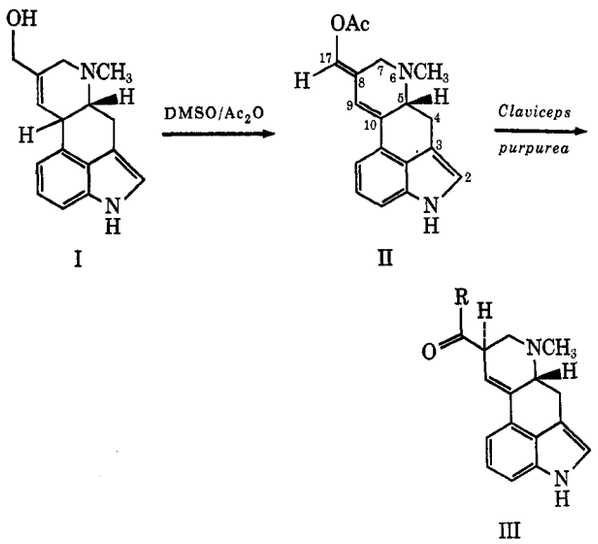
It is well known that simple clavine alkaloids, particularly agroclavine and elymoclavine (I), are precursors of the lysergic acid (III, R = OH) moiety of more complex amide and peptide type ergot alkaloids;[1-5]



however, the sequence of steps from I to the lysergic acid stage is unknown. Lysergene, lysergol, isolysergol, and penniclavine apparently are not precursors of lysergic acid derivatives.[2,3] Therefore, shift of the double bond into the 9,10 position is not the first step. 6-Methyl-8-ergolene-8-carboxylic acid ($\Delta^{8,9}$-lysergic acid), a natural constituent of certain ergot strains,[6] was found to be incorporated into lysergic acid amides, although not so efficiently as lysergic acid.[7] While this could indicate biological double bond isomerization at the lysergic acid stage, the fact that the same reaction also occurs spontaneously at a measurable rate[6] makes the interpretation of this experimental result somewhat ambiguous. In order to examine this question further, we attempted to prepare $\Delta^{8,9}$- and/or $\Delta^{9,10}$-lysergaldehyde (III, R = H) from elymoclavine. Surprisingly, it turns out that the hydroxymethyl group of elymoclavine is extremely resistant to most of the usual oxidizing agents. This fact has apparently been noted before in extensive attempts to produce lysergic acid commercially by chemical oxidation of elymoclavine.[8] The only defined oxidation products obtained were penniclavine and isopenniclavine, the products of hydroxylation in the 8 position.[9,10]

Treatment of elymoclavine with a mixture of dimethyl sulfoxide and acetic anhydride at room temperature for 12 hr[11] produced, in addition to elymoclavine *O*-acetate, a new compound which was identified as 6-methyl-8-acetoxymethylene-9-ergolene (II), the enol acetate of lysergaldehyde. Separation of the two

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