Acknowledgment. We express our deep gratitude to Masaaki Kurihara for experimental work in β-lactam formation.

24-Methyl-25-azacycloartanol, an Analogue of a Carbonium Ion High-Energy Intermediate, Is a Potent Inhibitor of (*S*)-Adenosyl-L-methionine:Sterol C-24-Methyltransferase in Higher Plant Cells

Acharan S. Narula*

Research School of Chemistry
Australian National University
Canberra, ACT 2600, Australia

Alain Rahier, Pierre Benveniste,* and Francis Schuber

Institut de Botanique, Laboratoire de Biochimie Végétale
67083-Strasbourg Cédex, France

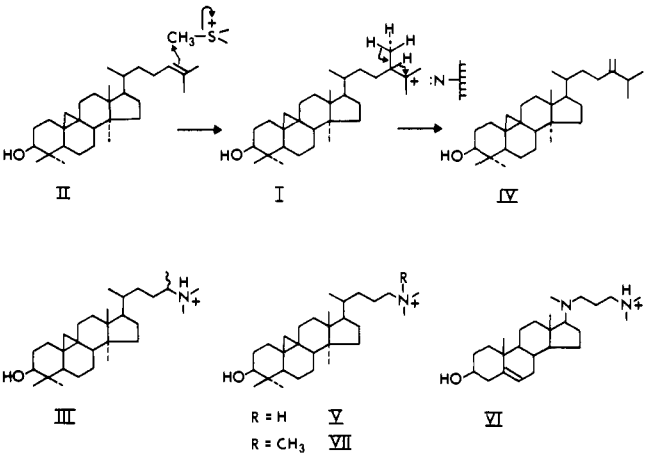
Received December 29, 1980

Theoretical considerations have established that catalysis of a reaction by an enzyme implies that the activated form of the substrate, occurring during the reaction pathway, is bound more energetically by the active site than the substrate in its ground state.[1,2] The result is that molecules which bear a structural and electronic resemblance to metastable intermediates should be very strong inhibitors of catalyzed reactions.[2,3]

Studies on (*S*)-adenosyl-L-methionine (SAM):sterol C-24-methyltransferases[4-7] have shown that the C-methylation reaction can be viewed as a nucleophilic attack by the Δ^{24} double bond of various sterols on the methyl group of the sulfonium group of SAM. This reaction leads to the formation of a high-energy intermediate (HEI, I) possessing a methyl at C-24 and a carbonium ion at C-25. After a hydride transfer from C-24 to C-25, an elimination of a proton at C-28 occurs giving a 24-methylene sterol (Scheme I). For an explanation of the stereochemical features of this reaction, it has been postulated that the C-25 carbonium ion could be stabilized by an electron-bearing residue of the active site of the enzyme, leading to a transient interaction of I with the enzyme.[6,8] With these considerations in mind, we thought that it was possible to mimic the carbonium ion of I by replacing the C-25 by a nitrogen atom in the structure of the intermediate. The resulting 25-aza derivative being essentially protonated under physiological conditions presents certain electronic similarities with the HEI (I), i.e., tetrahedral ammonium ion compared to the trigonal carbonium ion, and could therefore behave as a potent inhibitor of the methylation reaction. Since in photosynthetic eukaryotes cycloartenol (II) has been shown to be the best substrate in vivo and in vitro of the C-methylation reaction, (24-*RS*)-24-methyl-25-azacycloartanol (III) has been synthesized and assayed as an inhibitor of (*S*)-adenosyl-L-methionine:cycloartenol C-24-methyltransferase (CMT).

(24-*RS*)-24-Methyl-25-azacycloartanol was prepared as follows: To the known[9a] 3β-acetoxy-9β,19-cyclo-25,26,27-trinorlanostan-24-one (114 mg) taken in a 2:1 mixture of dry MeOH–THF (10 mL) was added 10 mL of methanol solutions of dimethylamine hydrochloride (stock solution was prepared by dissolving 2.17 g of $(CH_3)_2NH_2^+Cl^-$ in 50 mL of MeOH) and 60 mg of $NaCNBH_3$ under N_2 atmosphere. After stirring at room temperature for 72 h, the reaction mixture was diluted with water and the organic part was extracted in ether and dried (Na_2SO_4) and then treated with 40 mg of LAH for 4 h at room temperature. After conventional workup, 103 mg of 24-methyl-25-azacycloartanol (epimeric at C-24), mp 110–114 °C, 120–123 °C, was obtained. The assigned structure was in full agreement with its spectral data (NMR, IR, Mass).[9b]

Scheme I



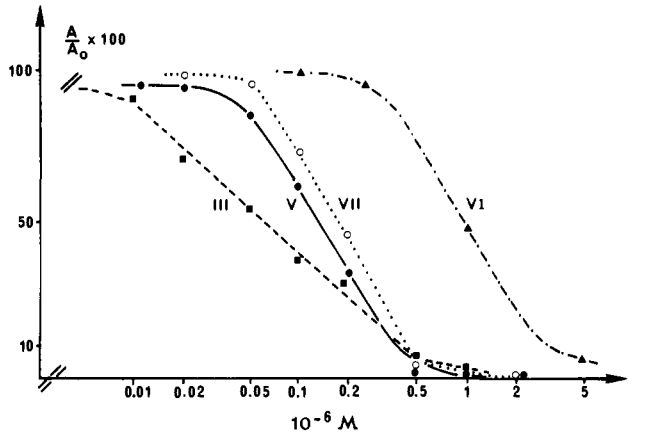


Figure 1. Inhibition of the (*S*)-adenosyl-L-methionine:cycloartenol C-24-methyltransferase by high-energy analogues: III (-■-■-), V (-●-●-), VI (-▲--▲--), and VII (··O··O··O··). A_0, A, activities measured, respectively, in the absence or presence of inhibitor, the concentration of substrate II being 100 μM.

Microsomes (0.5 mL) from maize seedlings were incubated in the presence of [*Methyl*-^{14}C]SAM (100 μM, 0.1 μCi), cycloartenol (100 μM), and various concentrations of III for 1 h at 30 °C and pH 7.4. A control which lacked III was incubated in parallel. The reactions were stopped by 6% methanolic KOH (1 mL), and the neutral lipids were extracted and analyzed as described.[10,11] The radioactivity incorporated in the 4,4-dimethyl sterol fraction was shown to be associated with 24-methylenecycloartanol (IV).[10,11] Figure 1 shows the inhibitory effect of III on the enzymatic activity. From the observed curve it was possible to calculate a I_{50} (inhibitor concentration required to reduce the reaction velocity by half) value of 0.05 μM. Under the assay conditions used in this study where the concentration of the substrate was close to its K_M value (100 μM), I_{50} values are of the order of the inhibition constants[12] and the value found for III indicates that CMT has a much higher affinity for the HEI analogue than for its substrate II. For an assessment of the inhibition specificity, the inhibitory power of III has been compared with that of 25-azacycloartanol (V) and 20,25-diazacholesterol (VI).[10,11] Results showed that III was more efficient than V to inhibit the CMT, while VI was the least potent compound. Thus III appeared to be the best among the inhibitors tested, and this

(1) Pauling, L. *Chem. Eng. News.* **1946**, *24*, 1375.
(2) Wolfenden, R. *Annu. Rev. Biophys.* **1976**, *5*, 271.
(3) Jencks, W. P. *Adv. Enzymol.* **1975**, *43*, 219.
(4) Nes, W. R. *Adv. Lipid Res.* **1977**, *15*, 253.
(5) Lederer, E. *Biochem. J.* **1964**, *93*, 449.
(6) Goad, L. J.; Goodwin, T. W. *Prog. Phytochem.* **1972**, *3*, 113.
(7) Arigoni, D. *Ciba Found. Symp.* **1978**, 243.
(8) Cornforth, J. W. *Angew. Chem., Int. Ed. Engl.* **1965**, *7*, 903.
(9) (a) Narula, A. S.; Dev. S. *Tetrahedron.* **1971**, *27*, 1119. (b) Complete details of the ^{1}H NMR spectra will be given in the full paper.
(10) Fonteneau, P.; Hartmann-Bouillon, M. A.; Benveniste, P. *Plant Sci. Lett.* **1977**, *10*, 147.
(11) Rahier, A.; Narula, A. S.; Benveniste, P.; Schmitt, P. *Biochem. Biophys. Res. Commun.* **1980**, *92*, 20.
(12) Chou, T. *Mol. Pharmacol.* **1974**, *10*, 235.

0002-7863/81/1503-2408$01.25/0

result was in full agreement with our hypothesis that III was electronically and stereochemically closest to the HEI (I). In order to check whether the neutral or the protonated amine was the inhibitory species, the derivative possessing a quaternary ammonium function VII was synthesized[9b] and used as inhibitor in the assay. The results (Figure 1) showed that VII was as strongly inhibitory as V, indicating that the charged species was probably the inhibitory one.

To our knowledge, this work demonstrates for the first time that a carbonium ion intermediate, involved in the biosynthesis of a sterol, can be mimicked by an ammonium group; such a result has important implications both in the search of new inhibitors and in our understanding of the molecular mechanisms involved in sterol biosynthesis.

As an example of the potential application of this class of compounds in vivo, III when given to bramble (*Rubus Fruticosus*) cell suspension growing in a liquid medium, was shown to inhibit drastically the sterol side chain methylation reactions resulting into a spectacular decrease of 24-ethyl sterols (major sterols in the control) and a correlative increase of 24-unalkylated sterols (not present in the control).[13] Thus III appears to be a molecular tool capable of manipulating in vivo the relative proportions of 24-methyl, 24-ethyl, and 24-unalkylated sterols in plant cells. Similarly 25-aza-24,25-dihydrozymosterol has recently been shown to block the C-24 methylation of zymosterol in *Saccharomyces cerevisiae*.[14,15]

(13) (a) Schmitt, P.; Benveniste, P., to be published. (b) The action of V on 24-alkyl sterol biosynthesis in bramble cells has been published: Schmitt, P.; Narula, A. S.; Benveniste, P.; Rahier, A. *Phytochemistry* **1981**, *20*, 197.

(14) Avruch, L.; Fischer, S.; Pierce, Jr., N.; Oehlschlager, A. C. *Can. J. Biochem.* **1976**, *54*, 657.

(15) Oehlschlager, A. C.; Pierce, H. D., Jr.; Pierce, A. M.; Angus, R. H.; Quantin-Martenot, E.; Unrau, A. M.; Srinivasan, R. In "Biogenesis and Function of Plant Lipids"; Mazliak, P., Ed.; Elsevier-North-Holland: Amsterdam, 1980; p 395.

(16) We are indebted to Professor R. E. Counsell (Medical School, The University of Michigan, Ann Arbor) for a kind gift of 20,25-diazacholesterol.

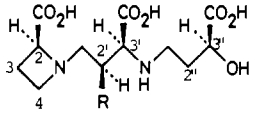
Total Synthesis of 2′-Deoxymugineic Acid, the Metal Chelator Excreted from Wheat Root

Yasufumi Ohfune,* Masako Tomita, and Kyosuke Nomoto

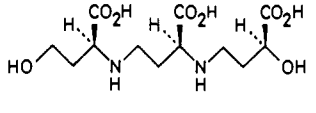
Suntory Institute for Bioorganic Research
Shimamoto-cho, Mishima-gun
Osaka 618, Japan

Received December 29, 1980

It is well-known that plants require many essential elements for their growth and maintenance; for example, iron is required in the biosynthesis of chlorophyll, and iron deficiency results in iron chlorosis.[1] Recently several amino acids possessing chelating properties for iron and other metals have been isolated from the root washing of gramineous plants grown under iron-deficient conditions.[2] Thus mugineic acid (**1**) has been isolated from barley



1, R = OH, **2**, R = H

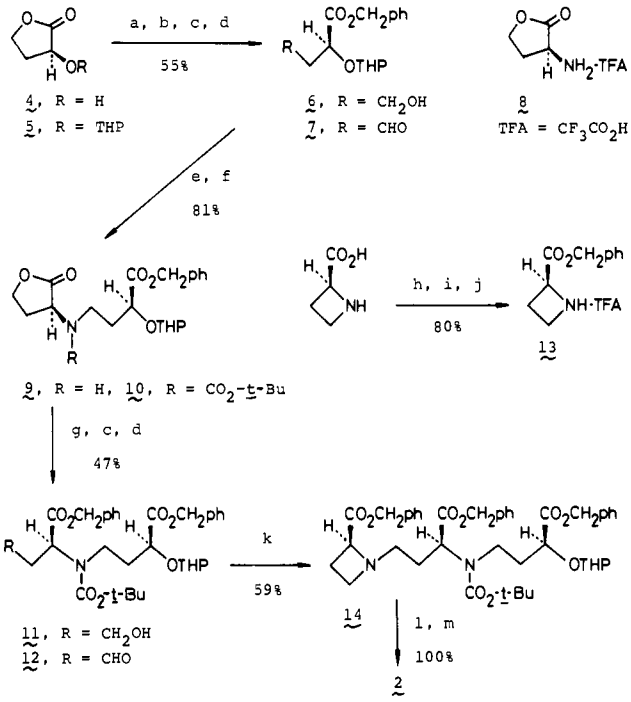


3

(*Hordium vulgare* L.),[3] 2′-deoxymugineic acid (**2**) from wheat (*Triticum aestivum* L.),[4] and avenic acid A (**3**) from oat (*Avena sativa* L.).[5] It was demonstrated that addition of either **1** or **2** to the medium of water-cultured rice at pH 7 increases the chlorophyll content;[6] thus it is considered that they are involved in the uptake and transport of iron (and other elements) in higher plants. Structure **2** (with undefined stereochemistry at C-3″) was suggested for 2′-deoxymugineic acid on the basis of degradative studies and NMR comparisons with mugineic acid (**1**) of established structure.[4,7] The syntheses of these metal chelators are of both practical and academic interest in view of their biological roles, the minute quantity available, and the presence of multiple water-solubilizing functionalities in their structures. We report here the total synthesis of natural (−)-2′-deoxymugineic acid (**2**) *which establishes the configuration of chiral centers as 2S, 3′S, 3″S* (as shown in **2**).

The structure of **2** reveals moieties derivable, either synthetically or biogenetically, from α-hydroxybutyric acid, homoserine, and the unique azetidine-2-carboxylic acid. Our synthetic plan for this molecule was to combine these three optically active units. The following crucial aspects had to be considered for the construction of 2′-deoxymugineic acid: (1) Only few methods exist for forming *N*-alkyl bonds in α-amino acids;[8] (2) mild reaction conditions are essential for each step to avoid racemization; (3) Selection of suitable protecting groups and deprotection at the

Scheme I[a]



[a] (a) DHP, TsOH, CH_2Cl_2, 0 °C, 30 min. (b) 2.5% KOH (1 equiv)–THF (1:1), room temperature, 1 h. (c) $PhCH_2Br$, 18-crown-6 (0.05 equiv), DMF–H_2O (4:1), room temperature, 6 h. (d) PCC, CH_2Cl_2, room temperature, 2 h. (e) **8**, $NaBH_3CN$ (1.0 equiv), 0 °C (1 h), room temperature, 16 h. (f) $(t\text{-BuOCO})_2O$, Et_3N (0.05 equiv), room temperature, 14 h. (g) 2.5% KOH (2 equiv)–dioxane (1:1), room temperature, 14 h. (h) Boc-ON, Et_3N, room temperature, 4 h. (i) 2.5% KOH (**1** equiv), $PhCH_2Br$, 18-crown-6, DMF. (j) CF_3COOH, CH_2Cl_2, room temperature, 30 min. (k) **13**, $NaBH_3CN$, MeOH, 0 °C (1 h), room temperature, 16 h. (l) H_2 (1 atm), 5% Pd–C, EtOH–H_2O (4:1), 1 N HCl (trace), 14 h. (m) CF_3COOH, room temperature, 1 h.

(1) Brown, J. C. *Annu. Rev. Plant Physiol.* **1956**, *7*, 171.

(2) Takagi, S. *Soil. Sci. Plant Nutr. (Tokyo)* **1976**, *22*, 423.

(3) Takemoto, T.; Nomoto, K.; Fushiya, S.; Ouchi, R.; Kusano, G.; Hikino, H.; Takagi, S.; Matsuura, Y.; Kakudo, M. *Proc. Jpn. Acad., Ser. B* **1978**, *54*, 469.

(4) Nomoto, K.; Yoshioka, Y.; Arima, M.; Takemoto, T.; Fushiya, Y.; Takagi, S. *Helv. Chim. Acta*, in press.

(5) Fushiya, S.; Sato, S.; Nozoe, S.; Nomoto, K.; Takemoto, T. *Tetrahedron Lett.* **1980**, 3071.

(6) Takagi, S., to be published.

(7) Metal chelation of mugineic acid was confirmed by X-ray crystallographic analysis using Cu^{2+} (1:1 complex): Nomoto, K.; Mino, Y.; Ishida, T.; Yoshioka, H.; Ota, N.; Inoue, M.; Takagi, S.; Takemoto, T. *J. Chem. Soc., Chem. Commun.*, in press.

(8) Sangster, A. W.; Thomas, S. E.; Tingling, N. L. *Tetrahedron* **1975**, *31*, 1135. Schellenbergh, K. A. *J. Org. Chem.* **1963**, *28*, 3259. Biemann, K. "Mass Spectrometry Organic Chemical Applications"; McGraw-Hill: New York, 1962; p 358.

0002-7863/81/1503-2409$01.25/0