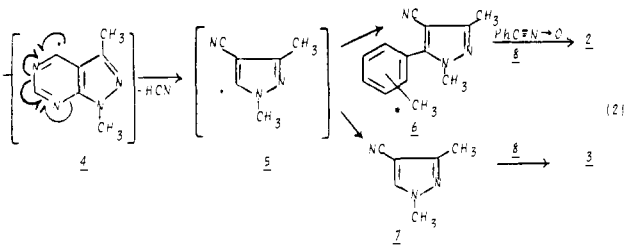

product **7**. Toluene in the presence of nitric oxide for extended periods of time may nitrosate or nitrate on the methyl group.[9] Subsequent oximation and thermal elimination of nitrous acid would form benzonitrile *N*-oxide (**8**). Electrocyclic addition of *N*-oxide **8** to nitriles **6** or **7** forms 1,2,4-oxadiazoles **2** or **3**.[10]

Support for the proposed origin of **2** and **3** was obtained by using 20% $^{13}CH_3$-enriched toluene as the reaction solvent. ^{13}C enrichment (indicated by an asterisk in eq 1 and 2) occurred at ring B carbon 3 as well as the C-methyl of ring D as predicted by the above mechanism (Table I).

The reasons that **4** collapses to **5** (and thus to products) rather than reacting to form the desired arylation product are not clear. Further work to explore the generality of this reaction and to prepare the desired 4-arylated pyrazolo[3,4-*d*]pyrimidines is the subject of a future report from these laboratories.[11]

Acknowledgment. We thank Drs. W. E. Gore and G. H. Birnberg for enlightening discussions and Professor A. S. Kende for his suggestions during preparation of this manuscript.

Registry No. 1, 5346-58-7; **2a**, 82044-26-6; **2b**, 82044-27-7; **3**, 82044-28-8.

Supplementary Material Available: Tables of atomic coordinates and thermal parameters for **2a** (2 pages). Ordering information is given on any current masthead page.

(9) For a discussion of aralkyl side-chain oxidations and nitrations under these conditions see: (a) Sosnovsky, G. "Free Radical Reactions in Preparative Organic Chemistry"; MacMillan: New York, 1964; pp 217-227. (b) Touster, O. *Org. React.* **1953**, *7*, 327-377.

(10) Chang, M. S.; Lowe, J. U., Jr. *J. Org. Chem.* **1967**, *32*, 1577-1579.

(11) Press, J. B.; Eudy, N. H., to be submitted for publication.

Identity of Cosynthetic Factor 1 of *Streptomyces aureofaciens* and Fragment FO from Coenzyme F420 of *Methanobacterium* Species

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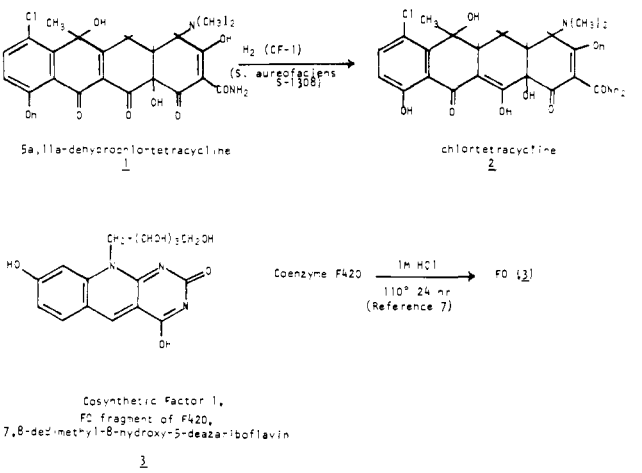
Received February 2, 1982

In the early part of our work on the biosynthesis of the tetracyclines, we isolated from *Streptomyces aureofaciens* W-5 a substance designated cosynthetic factor 1 (CF-1), which was involved as a catalyst in the biological reduction of 5a,11a-dehydrochlortetracycline (**1**) to chlortetracycline (**2**).[1] At that time, the available quantity of CF-1 was insufficient to permit a complete structure determination. We were able, however, to postulate a close relationship to the pteridines and flavins and noted that the properties of CF-1 did not correspond exactly to any of the then known members of those families.[1]

The identification of CF-1 as 7,8-didemethyl-8-hydroxy-5-deazariboflavin (**3**) has come about by our recent application of nuclear magnetic resonance methods to the study of the CF-1 structure. Carbon-13 NMR of CF-1 in dimethyl sulfoxide[2] clearly showed the presence of a ribityl side chain like that in riboflavin[2]



and indicated a total of 16 carbon atoms. Line broadening indicated that only four of the nonribityl carbons carried protons. Proton NMR clearly showed the presence of a 1,2,4-trisubstituted benzene ring fragment,[3] an additional isolated vinyl proton, two hydroxyl or imino protons, and eleven additional protons that could be accounted for by the ribityl side chain. Thus, 17 protons were seen, and this $C_{16}H_{17}$ partial formula, when taken with our originally reported microanalysis, suggested the molecular formula: $C_{16}H_{17}N_3O_7 \cdot H_2O$.[4] Confirmation of this composition was found in the chemical ionization negative-ion mass spectrum of CF-1, which showed a strong molecular ion, M^- 363, and the fragment ions M^- 345 (loss of H_2O), M^- 320 (loss of CONH), and M^- 229 (loss of $C_5H_{10}O_4$).

At the time of our orignial work, vigorous acidic hydrolysis had yielded microgram quantities of a product having ultraviolet absorption spectra reminiscent of a hydroxycarbostryl,[5] and this, with our more recent data, above, suggested a possible deazariboflavin structure. A literature search revealed the intervening work on coenzyme F420. This coenzyme has been implicated both in hydrogen transfer in the production of methane by several species of *Methanobacterium*[6,7] and also as a cofactor in a photoreactivating system of *Streptomyces griseus*.[8] From the very characteristic absorption spectra[9] of CF-1 and coenzyme F420, it was evident that we were dealing with a closely related compound. It appeared likely that CF-1 was identical with the FO fragment derived hydrolytically from F420, and the very close agreement between some proton magnetic resonance shifts[3] of CF-1 and synthetic FO left little doubt that the identification was correct. The exact correspondence of the infrared spectra[10] of CF-1 and a sample of synthetic FO and the agreement of optical rotations of the two materials[11] confirmed the identity of the compounds.

(1) Philip A. Miller, Newell O. Sjolander, Stephen Nalesnyk, Nancy Arnold, Sylvia Johnson, Albert P. Doerschuk, and J. R. D. McCormick, *J. Am. Chem. Soc.*, **82**, 5002 (1960).

(2) ^{13}C NMR of CF-1 (20 MHz, Me_2SO-d_6) δ 164.1, 162.3, 157.9, 156.4, 143.9, 141.3, 133.6, 115.2 (2 C), 110.5, 102.0, 73.7, 72.6, 69.4, 63.2, 47.8. ^{13}C NMR of the ribityl carbons of riboflavin (Me_2SO-d_6) δ 73.5, 72.6, 68.7, 63.2, 47.1.

(3) ^1H NMR of CF-1 (80 MHz, Me_2SO-d_6) (partial spectrum) δ 11.23, 10.98, 8.94, 8.05 (d, J = 10 Hz), 7.41, 7.06 (d, J = 10 Hz). ^1H NMR of FO (ref 12) (60 MHz, Me_2SO-d_6) (partial spectrum) δ 11.2 (br), 11.01, 8.89, 8.01 (d, J = 9 Hz), 7.40, 7.04 (d, J = 9 Hz).

(4) In ref 1 we reported the composition as $C_{19}H_{22}N_4O_7$, based on an erroneous neutral equivalent of 446.

(5) Unpublished work with E. R. Jensen of these laboratories.

(6) P. Cheeseman, A. Toms-Wood, and R. S. Wolfe, *J. Bacteriol.*, **112**, 527 (1972).

(7) L. Dudley Eirich, Godfried D. Vogels, and Ralph S. Wolfe, *Biochemistry*, **17**, 4583 (1978).

(8) A. P. M. Eker, A. Pol, P. vanden Meyden, and G. D. Vogels, *FEMS Microbiol. Lett.*, **8**, 161 (1980).

(9) See Figure 1 in ref 6 and Figure 2 in ref 1.

(10) The infrared spectra of our material and that of the sample supplied by Professor Walsh were initially similar but not identical. After converting each sample to its ammonium salt and back to its free acid form, the major features of both were identical: IR (max) (KBr) 3400, 3160, 3050, 2790, 1735, 1710, 1625, 1585, 1495, 1450, 1395, 1340, 1275, 1225, 1150, 1115, 1090, 1065, 1030, 910, 845, 815, 795, 750, 680, 580, 550, 500, 425 cm^{-1}.

(11) Optical rotations, CF-1: $[\alpha]^{25}_D$ +40 ± 2° (0.5% in 0.1 N NaOH); FO (ref 12): $[\alpha]^{25}_D$ +38° (0.5% in 0.1 N NaOH).

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Acknowledgment. We thank Nancy Arnold Perkinson of Lederle Laboratories for the chemical ionization mass spectrum and Professor Christopher Walsh of Massachusetts Institute of Technology for kindly supplying a reference sample of synthetic FO.[12]

Registry No. 3, 37333-48-5.

(12) Wallace T. Ashton, Ronald D. Brown, Frederick Jacobson, and Christopher Walsh, *J. Am. Chem. Soc.*, **101**, 4419 (1979).

Total Synthesis of Milbemycin β_3

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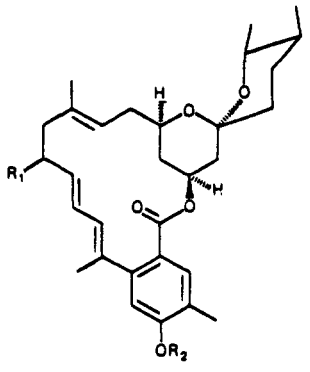
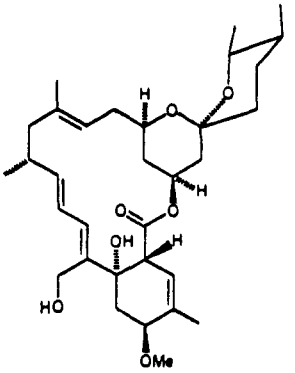
Received April 6, 1982

Milbemycin β_3 (**1a**), the simplest member of a family of some 13 architecturally novel macrolide antibiotics structurally related to the avermectins,[2] was first isolated in 1975 by Mishima et al.[3,4] from *Streptomyces* B-41-146. Subsequent screening demonstrated that this antibiotic complex possessed remarkably potent pesticidal activity[4] against a host of agricultural pests, including aphids, laval forms of insects of the order *Lepidopera*, mites, rice leaf beetles, and tent caterpillars, with little or no associated phytotoxicity.[4]



1



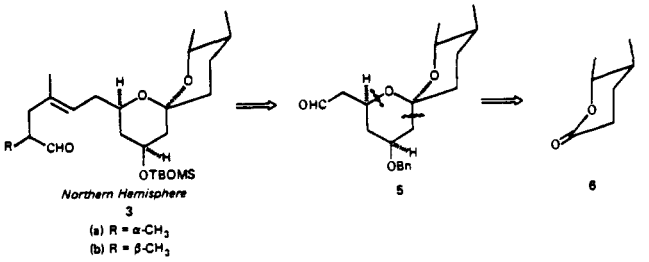
2

(a) R_1 = α-CH_3; R_2 = H
(b) R_1 = β-CH_3; R_2 = H
(c) R_1 = α-CH_3; R_2 = CH_3
(d) R_1 = β-CH_3; R_2 = CH_3

The structures of the milbemycins, initially assigned on the basis of detailed spectroscopic analysis, were secured through aegis of a single-crystal X-ray analysis of milbemycin β_1 (**2**).[3] Central to the derived structures are the spiroketal functionality, the 16-membered macrolide ring, and the conjugated diene system.

In this communication we announce preparation of milbemycin β_3 (**1a**), *the first member of the milbemycin–avermectin class to yield to total synthesis.* We note in advance that our strategy is short (longest linear sequence, 16 steps), convergent, and highly stereocontrolled.[5,6]

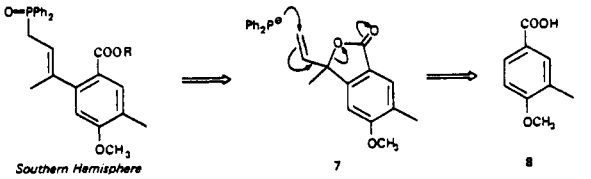
At the onset, we set as an overall goal the development of a common synthetic strategy that would yield members of both the milbemycin and avermectin families as well as possible structural analogues of biological interest. From the retrosynthetic perspective we initially divided milbemycin β_3 at the diene and ester linkage to generate the spiroketal northern hemisphere (**3a**) and



Northern Hemisphere
3
(a) R = α-CH_3
(b) R = β-CH_3

5

6



Southern Hemisphere
4
(a) R = H
(b) R = CH_3

7

8

an aromatic southern hemisphere (**4**). Union of the two was envisioned to take place via a Horner–Wittig coupling[7] followed by macrocyclic lactonization.[8] Further simplification of the northern hemisphere lead to aldehyde **5**, which in turn could be derived from lactone **6**.[9] The aromatic southern hemisphere, on the other hand, was envisioned to arise via a novel S_N2' displacement employing lithium diphenylphosphide[10] on lactone **7**, the latter prepared from 3-methyl-*p*-anisic acid (**8**).[11]

The success of this scenario rested on our ability to construct aldehyde **5** in a stereocontrolled manner. Here we planned to take advantage of the anomeric effect.[12] In particular, under equil-

(1) Camille and Henry Dreyfus Teacher Scholar, 1978–1983; National Institutes of Health (National Cancer Institute) Career Development Awardee, 1980–1985.

(2) Albers-Schönberg, G.; Arison, B. H.; Chabala, J. C.; Douglas, A. W.; Eskola, P.; Fisher, M. H.; Lusi, A.; Mrozik, H.; Smith, J. L.; Tolman, R. L. *J. Am. Chem. Soc.* **1981**, *103*, 4216. Springer, J. P.; Arison, B. H.; Hirshfield, J. M.; Hoogsteen, K. *J. Am. Chem. Soc.* **1981**, *103*, 4221.

(3) Mishima, H.; Kurabayashi, M.; Tamura, C. *Tetrahedron Lett.* **1975**, 711.

(4) Takiguchi, Y.; Mishima, H.; Okuda, M.; Terao, M.; Aoki, A.; Fukuda, R. *J. Antibiot.* **1980**, *33*, 1120.

(5) A preliminary account of this work, in which completion of the first total synthesis of milbemycin β_3 was disclosed, was presented at the 183rd National Meeting of the American Chemical Society, Las Vegas, NV, March 1982, ORGN 16.

(6) To the best of our knowledge there exists only two other reports directed at construction of the basic carbocyclic ring of the milbemycin; see: Attwood, S. V.; Barrett, A. G.; Florent, J.-C. *J. Chem. Soc. Chem. Commun.* **1981**, 556. Williams, D. R.; Barner, B. A.; Phillips, J. G.; Nishitani, K., 183rd National Meeting of the American Chemical Society, Las Vegas, NV, March 1982, ORGN 15.

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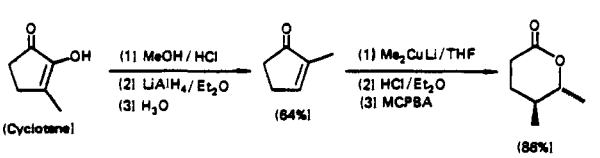
(9) Lactone **6** has been prepared in both racemic and chiral form, the latter from (−)-citronellol. The racemic approach beginning with cyclotene is illustrated below. For other approaches to lactone **6** see: Dev, S.; Rai, C. *J. Ind. Chem. Soc.* **1957**, *34*, 266. Honkanen, E.; Moisio, T.; Karvonen, P.; Virtanen, A. I. *Acta Chem. Scand.* **1968**, *22*, 2041.



(Cyclotene)
(64%)
(86%)

(10) Mann, F. G.; Tong, B. P.; Wystrach, V. P. *J. Chem. Soc.* **1963**, 1155. Mann, F. G.; Pragnell, M. J. *J. Chem. Soc.* **1965**, 4120. Ireland, R. E.; Welch, S. C. *J. Am. Chem. Soc.* **1970**, *92*, 7232.

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(12) For a discussion on the utility of the oxygen anomeric effect in the construction of architecturally complex natural products see: Kishi, Y. *Lect. Heterocycl. Chem.* **1980**, *5*, S95.

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