A Stereospecific Total Synthesis of (±)-Methylenomycin A and Its Epimer, (±)-Epimethylenomycin A

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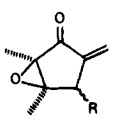
Abstract: A stereospecific total synthesis of the racemates of methylenomycin A and its epimer, epimethylenomycin A, has been achieved. These novel cyclopentanoid antibiotics were constructed in eight and ten steps, respectively, utilizing a common intermediate (**14**) without resort to protecting-group methodology. The key elements of the total synthesis were (i) stereospecific introduction of the epoxide group syn and anti in common intermediate **14**; (ii) introduction of the carbonyl at C-6 in the furan rung of **19** and **20** via ruthenium tetroxide oxidation; (iii) retrolactonization of lactones **12** and **13** through agency of lithium thiomethoxide. The overall efficiencies of this approach to methylenomycin A and its epimer, based on maleic anhydride, were 12 and 4%, respectively. In addition, new synthetic methodology based on organoselenium chemistry was developed.

Introduction and Background

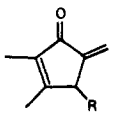
We wish to record here a full account of the first total synthesis of the novel antibiotic (±)-methylenomycin A (**1**),[2,3] as well as its epimer, (±)-epimethylenomycin A (**2**). We note in advance that our synthetic route is efficient (i.e., proceeds in 12 and 4% overall yield, respectively), affords both stereospecifically from a common intermediate without resort to protecting-group methodology, and in the case of methylenomycin A was effected without aid of chromatographic separation. Finally, two new synthetic methods based on organoselenium chemistry were developed during the course of this work.[4,5]

Methylenomycin A, a crystalline solid available from the culture broth of *Streptomyces violaceruber*, was shown by Haneishi in 1974 to process structure **1**;[6] the assignment was based on a direct X-ray crystallographic analysis.[7] A second antibiotic, methylenomycin B, also available from *Streptomyces violaceruber* and tentatively assigned structure **3** by Haneishi[6] on the basis of spectral comparisons with **1**, was later shown by total synthesis to possess structure **4**.[8] More recently, a closely related antibiotic, desepoxy-4,5-didehydromethylenomycin A (**5**), was isolated from *Streptomyces coelicolar A3(2)*,[9] a bacterium also known[10] to produce methylenomycin A. Indeed, **5** was shown to be a biosynthetic precursor of **1** via radio-labeling experiments.[9]

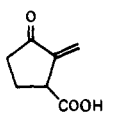
The methylenomycins (**1**, **4**, and **5**) represent three new members of a small but rapidly growing family of antibiotics,



1: R = α-COOH
2: R = β-COOH
3: R = H



4: R = H
5: R = COOH



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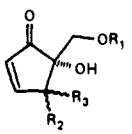
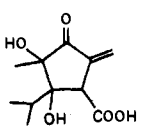


7a: R_1 = H, R_2 = α-OH, R_3 = H
b: R_1 = H, R_2 = α-OAc, R_3 = H
c: R_1 = Ac, R_2 = α-OH, R_3 = H
8a: R_1 = H, R_2 = β-OH, R_3 = H
b: R_1 = H, R_2 = β-OAc, R_3 = H
c: R_1 = Ac, R_2 = α-OH, R_3 = H
9a: R_1 = H, R_2 = R_3 = O



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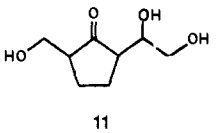

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all of which possess a cyclopentanone ring. This family, termed by us the cyclopentanoid antibiotics, now includes sarkomycin (**6**),[11] the pentenomycins I-III (**7a–c**),[12] the epipentenomycins I-III (**8a–c**),[12] dehydropentenomycin I (**9**),[13] xanthocidin (**10**),[14] and vertimycin (**11**).[15,16]

Our interest in methylenomycin A as a synthetic target was prompted by its novel structure, by its demonstrated in vitro activity against Gram-positive and Gram-negative bacteria, and by its similarity to the pharmacologically important α-methylene lactones[17] and to sarkomycin (**6**), a known antitumor agent.[11]

Results

(i) **A Strategy for the Stereospecific Total Synthesis of Methylenomycin A and Its Epimer, Epimethylenomycin A, from a Common Intermediate.** At the outset the highly functionalized nature of methylenomycin A placed two constraints on our synthetic strategy. First, it appeared prudent to introduce the highly reactive and possibly unstable α-methylene substituent[2] late in our synthetic scheme. Second, to ensure the requisite stereochemistry of methylenomycin A, a method was required which would allow the stereospecific introduction of the epoxide trans to the carboxyl group. With these constraints in mind, lactone **12**, an isomer of methylenomycin A, termed cyclomethylenomycin A, appeared to be an ideal penultimate precursor, in that the γ-butyrolactone could serve, via a direct retrolactonization process, as a latent equivalent of the required α-methylene-β-carboxyl functionality.

Should our synthetic scheme also provide access in stereospecific manner to the epimeric epoxylactone **13** (i.e., epicyclomethylenomycin A), a similar retrolactonization process would then afford the epimer of methylenomycin A. Taken in conjunction these considerations overwhelmingly suggested enone **14** to be an ideal *common* intermediate for construction of both methylenomycin A and its epimer, since the concave–convex nature of this bicyclic ketone would provide in both cases the needed geometric bias required to effect stereospecific introduction of the epoxide. This scenario, of course, assumes the successful and hopefully regioselective introduction of a carbonyl group at C-6 in the furan ring.

(ii) **Construction of the Initial Synthetic Target: Common Intermediate 14.** Our approach to enone **14** begins with the readily available cyclobutene **15** and is outlined in Scheme I. Taking advantage of the known[18] propensity of maleic anhydride to participate effectively in [2 + 2] photochemical cycloaddition reactions with acetylenes, irradiation with 2-pentyne in acetonitrile employing benzophenone as sensitizer

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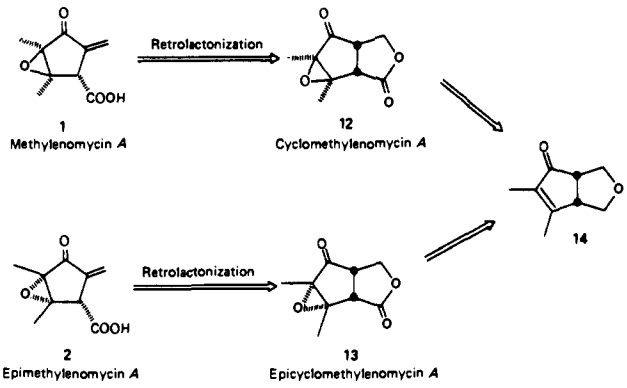
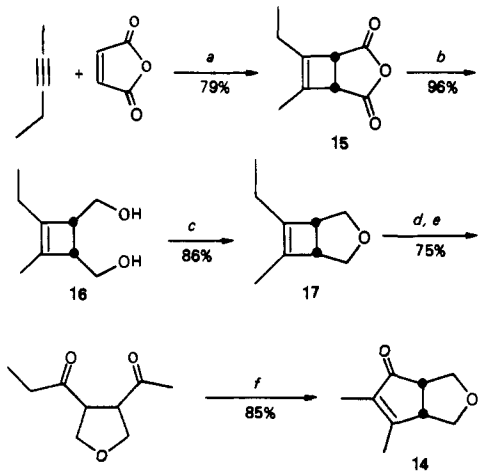


afforded **15** in 79% yield as a colorless oil after distillation. This photochemical [2 + 2] cycloaddition was most conveniently carried out on a 15–20-g scale employing the standard Hanovia 450-W mercury arc fitted with a Corex filter. Subsequent reduction of **15** with lithium aluminum hydride at the reflux point of tetrahydrofuran gave the desired diol (**16**) in excellent yield (96%). Diol **16** in turn was transformed to **17** in 86% yield by treatment with 1.1 equiv of *p*-toluenesulfonyl chloride in dry pyridine, first at 0 °C and then at reflux for several hours.[19] All synthetic intermediates in this study have been fully characterized; for those not discussed in detail, structural assignments rest on spectroscopic properties and elemental composition data recorded in the Experimental Section.

Elaboration of the cyclopentenone system as required in our initial synthetic target (**14**) was envisioned to involve expansion of the cyclobutene ring in **17** via oxidative cleavage of the olefinic bond and subsequent aldol condensation of the resultant unsymmetrical 1,4-diketone. That the aldol condensation would in fact take place to afford the desired disubstituted cyclopentenone, as opposed to the alternate monosubstituted enone, was assured by the recent work of McCurry and Singh.[20] Toward this end, ozonolysis of **17** at −78 °C in methanol followed by reductive cleavage of the ozonide with triphenylphosphine and distillation afforded the desired diketone (**18**) in 75% yield. This diketone was then subjected to the McCurry–Singh cyclization protocol; isolation afforded **14** in 85% yield as a beautifully crystalline solid melting at 75–76 °C. The structure of **14** was derived from its spectroscopic properties; in particular, the high-field (220 MHz) NMR spectrum revealed two three-proton singlets at 1.66 and 2.03 ppm indicative of vinyl methyl substituents,[21] while the infrared spectrum displayed characteristic cyclopentenone

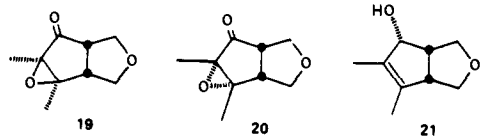
Scheme I



(*a*) *hν*/MeCN/Ph_2CO sens.; (*b*) $LiAlH_4$/THF, Δ; (*c*) TsCl/pyr. 0° C→reflux temp.; (*d*) O_3/MeOH/−78° C; (*e*) Ph_3P/MeOH; (*f*) 2% NaOH/95% MeOH.

absorptions[22] at 1700, 1655, and 920 cm^{-1}. The overall yield of enone **14**, based on maleic anhydride, was 42%.

(iii) **Stereospecific Epoxidation of 14.** With the initial success of our approach to **14** in hand, our attention next turned to the stereospecific introduction of the epoxide. Given the concave–convex nature of enone **14**, a basic hydrogen peroxide epoxidation protocol was expected to lead to **19** via addition to the least hindered or convex surface. The resultant epoxide (i.e., trans to the tetrahydrofuran ring) would then possess the stereochemistry required for methylenomycin A. To our delight, epoxidation of **14**, employing the conditions of Corey and Ensley,[23] yielded a *single* epoxy ketone (**19**) in 90% yield.



To establish rigorously the stereochemical outcome of this event, and furthermore to provide a viable approach to the epimeric epoxy ketone **20** for future elaboration of epimethylenomycin A (**2**), we again took advantage of the bicyclic nature of **14**. More specifically, 1,2 reduction of enone **14** with the sterically encumbered reducing agent diisobutylaluminum hydride[24] afforded endo alcohol **21** in 94% yield as the *sole* product. Epoxidation of this alcohol with *m*-chloroperbenzoic acid, a reagent known[25] to be directed by allylic hydroxyl substituents, followed by Jones oxidation[26] of the resultant mixture of epoxy alcohols afforded a 95:5 mixture (VPC) of two ketones, the major being a new crystalline epoxy ketone **20** (mp 65 °C) and the minor being **19**, identical in all respects (IR, 60- and 220-MHz NMR, and VPC retention time) with that prepared previously. Interestingly, attempts to effect separation and purification of the mixture of epoxy alcohols by vapor-phase chromatography (12.5% OV-101 at 170 °C) led efficiently to elimination of water; the resultant product, enone **14**, presumably arises via acid-catalyzed opening of the epoxide ring with concomitant hydride migration and then loss of water.[27]

The availability of both **19** and **20**, in conjunction with their mode of synthesis and spectroscopic properties, established the relative stereochemistry in each case. In addition, a highly stereoselective method was now available for the conversion of our "common intermediate" (**14**) to both **19** and **20** as required for independent synthesis of methylenomycin A (**1**) and its epimer (**2**).

(iv) **Introduction of a Carbonyl Group: a Key Transformation.** At this juncture our synthetic strategy called for the regioselective introduction of a carbonyl group at C-6 in the tetrahydrofuran ring of **19** and **20**. This task would have met with complete failure were it not for the known ability of ruthenium tetroxide to effect the oxidation of cyclic ethers to lactones.[28] Indeed, treatment of **19** with ruthenium tetroxide–sodium periodate, employing a two-phase catalytic protocol, afforded a *single* lactone (**12**).

Several comments concerning this transformation are in order. First, while apparently regioselective, the yield of **12** was consistently in the range of 45–47%. Furthermore, for complete conversion, a substantial excess of $NaIO_4$ (ca. >3 equiv) was required. Finally, while there was no question that we had in fact succeeded in effecting the desired introduction of a carbonyl, since the infrared spectrum displayed characteristic γ-lactone absorption[22] at 1775 cm^{-1}, the regiochemical outcome of this transformation as well as the reason for the observed selectivity remained open concerns.

To answer these questions, our attention turned to model studies. Three simple tetrahydrofurans (**22–24**) were chosen for this purpose. In each case $RuO_4/NaIO_4$ oxidation afforded the indicated lactones (**25–27**); the identity of each was es-

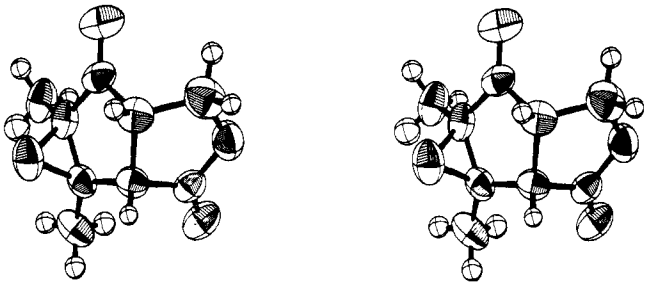
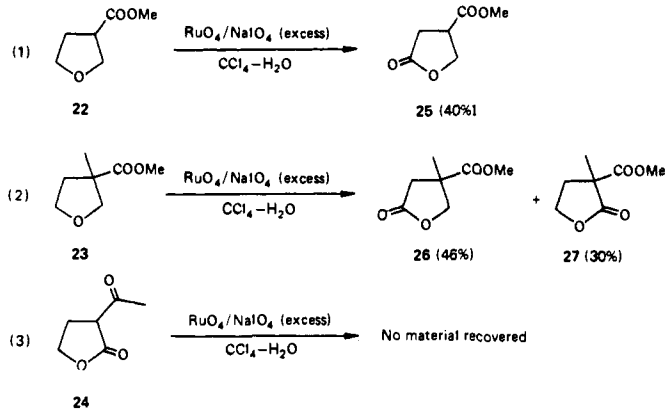


Figure 1. ORTEP stereoplot using 50% probability thermal ellipsoids for nonhydrogen atoms.



tablished by direct comparison with authentic samples. Taken together these observations suggest that the RuO_4 oxidation of **19** is in fact not regiospecific, but simply involves oxidative destruction of the alternate regiochemical product. That is, 1,3-dicarbonyl systems capable of enolization are oxidatively destroyed by RuO_4 (eq 3), while systems incapable of enolization (eq 2) are stable in further oxidation.

Although these observations provide strong support for the structure proposed for keto lactone **12,** the centrality of this intermediate to our approach to methylenomycin A required a rigorous assignment. Toward this end, we undertook and completed a single X-ray crystallographic analysis of (±)-cyclomethylenomycin A; the result of this study, already on record,[29] is illustrated in Figure 1.

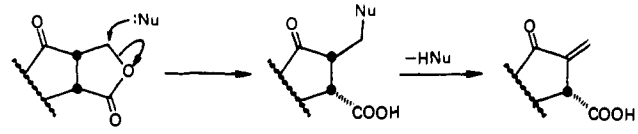
With the structure of cyclomethylenomycin A (**12**) secure, the epimeric epoxy ketone **20** was transformed without event to epicyclomethylenomycin A (**13**) employing the same $RuO_4/NaIO_4$ two-phase protocol. Again, a single crystalline lactone (mp 106.5–107.5 °C) was obtained in *46%* yield. The similar spectroscopic properties of **13** compared to those of cyclomethylenomycin A (**12**) clearly indicated that the two lactones were diastereomeric.

(v) Retrolactonization: the Cornerstone of Our Synthetic Strategy. With the synthesis of cyclomethylenomycin A (**12**) and epicyclomethylenomycin A (**13**) achieved, it remained only to unravel the latent α-methylene-β-carboxyl functionality concealed in the γ-lactone to complete the synthesis of **1** and **2.** As previously described we initially envisioned a direct retrolactonization process, possibly induced via the ketone enol or enolate.[30] Unfortunately, all attempts to effect this trans-

formation via either kinetic or thermodynamic deprotonation, employing such bases as lithium diisopropylamide, potassium and sodium hydride, potassium *tert*-butoxide, or DBN, led under a wide variety of solvent systems and temperature regimes only to recovery of starting material or to complex reaction mixtures. Furthermore, all attempts to effect the hydrolysis of the γ-butyrolactone through the agency of either acid or base catalysis were likewise thwarted, presumably by facile relactonization of the initially derived *cis*-γ-hydroxycarboxylic acid, prior to or during attempted isolation.[31]

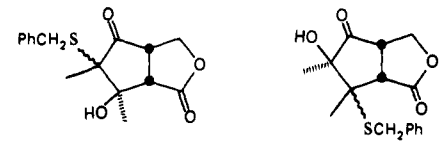
Shortly after initiation of our efforts to effect the desired retrolactonization, Baldwin pointed out[32] that such reactions, formally retro-5-endo-trigonal cyclizations, were in fact disfavored processes. While these rules for ring closure do not preclude such a transformation, especially under forcing conditions, it appeared in view of the potential instability of methylenomycin A that an alternative method for unraveling the latent functionality in the butyrolactone ring might be more suited to our purpose.

An attractive possibility appeared to be nucleophilic cleavage of the alkyl-oxygen bond of the lactone induced via a nonbasic nucleophile.[33] Subsequent elimination of the nucleophile would then yield methylenomycin A. Particular attention here was directed at devising reaction conditions wherein expulsion of the intially introduced nucleophile could be effected under mild conditions. Ideal candidates for this purpose appeared to be sulfur and selenium anions; mild oxidative-elimination[34] would then lead directly to methylenomycin A.



With this strategy in mind, we first examined the reaction of cyclomethylenomycin A (**12**) with a sulfur nucleophile.[35] To our delight, room temperature treatment with 1.5 equiv of benzylthiol and 2.0 equiv of DBN in benzene for 18 h led in near-quantitative yield to a crystalline solid (mp 188.5–189.5 °C), which by elemental composition and mass spectrometric data was shown to be a 1:1 adduct. Analysis of the infrared spectrum, however, revealed that the desired reaction had not occurred; instead cleavage of the epoxide ring had taken place. Epicyclomethylenomycin A (**13**) also afforded a similar crystalline 1:1 adduct (mp 154.5–155.5 °C), albeit in only 52% yield. Again the infrared spectrum indicated opening of the epoxide ring. No specific information about the regio- or stereochemistry of these adducts could be deduced from either the high-field (220 or 360 MHz) ^1H or ^{13}C NMR spectra, although the spectral data strongly suggested that the adducts were epimeric.

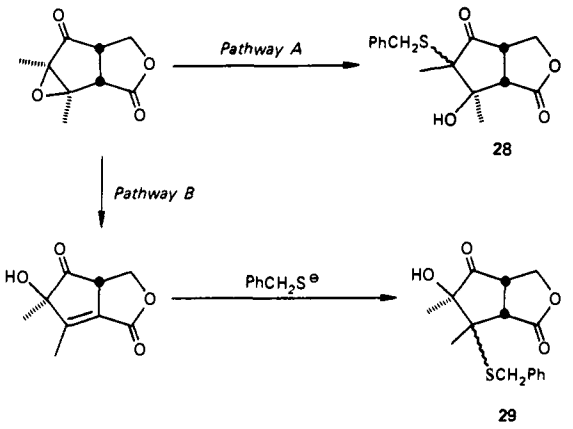
For the purposes of discussion, consider for the moment the regiochemical outcome of adduct formation; only two structures, **28** and **29,** are possible. Interestingly, feasible reaction pathways (i.e., A and B) leading to both regioisomers can be



28 **29**

envisioned. For example, there is ample precedent[36] that simple α,β-unsaturated epoxy ketones undergo efficient nucleophile attack at the α carbon of epoxy ketones. Such a transformation in the case of cyclomethylenomycin A (**12** → **28**), however, seems implausible since it would require that approach of the sulfur nucleophile occur on the concave surface of the bicyclic system, and furthermore that displacement take place at a quaternary center. Alternatively, base-induced elimination of the β-carbon-oxygen bond of the epoxide initiated by the lactone enolate could lead to a strained, and thereby highly reactive, α,β-unsaturated system which in a subsequent step

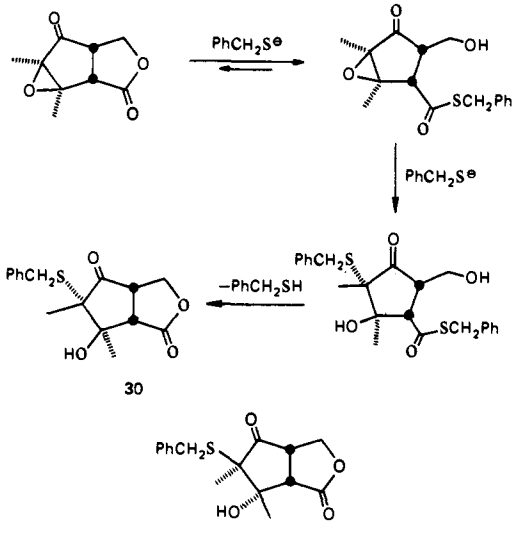
could undergo conjugate addition of thiolate anion to give **29.**



To define the structural requirements for this transformation, we subjected the epimeric epoxy ketones **19** and **20** to the same reaction conditions; in both cases a quantitative recovery of starting material resulted. This result, which demonstrates the obligatory nature of the carbonyl group, was quite consistent with our prejudice against pathway A, requiring concave approach of the thiolate anion.

Since conventional spectroscopic analysis of either adduct did not produce the required regio- or stereochemical information for structural assignment, an X-ray crystallographic analysis of the adduct derived from cyclomethylenomycin A was completed; the result of this study is illustrated in Figure 2.[37] To our surprise, addition had in fact occurred at the α center.

Confronted by this fact and the obligatory nature of the lactone carbonyl, we reconcile these observations by suggesting that adduct formation, at least in the case of cyclomethylenomycin A, occurs by initial opening of the lactone system, this mediated by attack of benzyl thiolate on the lactone carbonyl;[38] subsequent nucleophilic attack at the α center of the α,β-epoxy ketone would then not require an endo approach. Lactonization would then afford **30** as the observed adduct. Finally, we assign structure **31** to the adduct arising from epicyclomethylenomycin A on the basis of chemical analogy and close similarity of its spectroscopic properties to those of **30.**



(vi) New Synthetic Methodology: a Fruitful Detour into Organoselenium Chemistry. With attempts to effect the final conversion of **12** to methylenomycin A frustrated by the propensity of benzyl thiolate to induce epoxide cleavage, we next considered the more powerful and less basic nucleophile phenylselenolate. The reported nucleophile opening of γ- and δ-lactones with methyl-,[39] phenyl-,[40] and benzyl-[41] selenolates, albeit in only very modest yield, were quite intriguing.[42]

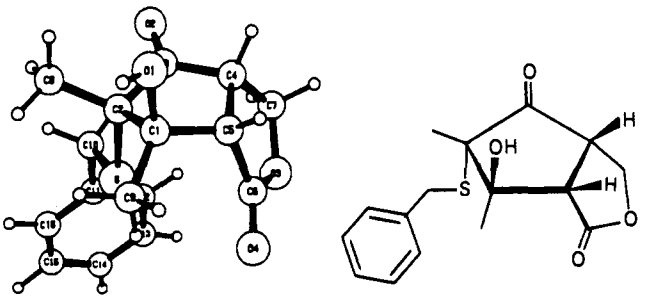
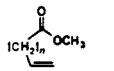
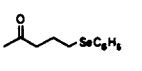
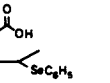
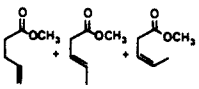


Figure 2.

Table I. A General Synthesis of ω-Olefinic Methyl Esters

	Substrate	Reaction time (hours)	Phenyl selenide	Yield (percent)	ω-Olefinic methyl ester	Yield (percent)
1	 $n = 1$	2		98		70
2	$n = 2$	3		96		65
3	$n = 3$	4		90		71
4	$n = 4$	10		90		71
5	$n = 9$	15		56		61
6		2		92		60
7		4		80	 3 : 3 : 1	78
8		20	No reaction			
9		20	No reaction			

With the hope of both extending and improving upon the previous experience with these reagents, we examined a number of model systems.[43] Sodium phenylselenolate was chosen for this study owing to its ready availability from commercially available diphenyl diselenide through sodium borohydride reduction in carefully deoxygenated DMF.[44] The results of this study are illustrated in Table I. In particular, we found that a wide variety of simple lactones suffer efficient alkyl–oxygen bond cleavage upon treatment with sodium phenylselenolate. Best results were obtained in DMF at 120–125 °C.

Several points concerning these transformations are in order. First, the efficiency of the reaction as well as the time required for complete conversion was substrate dependent. In general, the larger the lactone the longer the reaction time required to effect ring cleavage. Second, ring opening of 2-acetylbutyrolactone proceeded with concomitant loss of carbon dioxide to yield the corresponding ketone. Third, in the case of γ-lactones, even when the site of nucleophilic displacement was secondary, ring opening proceeded efficiently; tertiary γ-lactones and secondary δ-lactones, on the other hand, were inert. This selectivity may well find utility in natural-product synthesis.

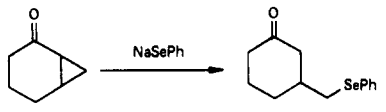
To complete the model study there remained only the oxidative elimination of the derived phenyl selenide. No difficulty was anticipated here in view of the now numerous examples of oxidative elimination of the phenyl selenide functionality.[35] To our surprise, however, all attempts to effect this transformation in the presence of a carboxyl group failed.[45] The corresponding methyl esters (CH_2N_2), on the other hand,

Table II. 220-MHz NMR Data of (±)-Methylenomycin A (1) and (±)-Epimethylenomycin A (2) (δ)

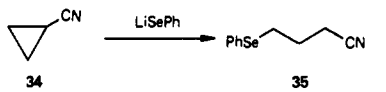
(±)-methylenomycin A (**1**): 1.48 (s, 3H), 1.58 (s, 3 H) 3.82 (m, 1 H) 5.65 (d, J = 1.8 Hz, 1 H), 6.27 (d, J = 1.8 Hz, 1 H)
(±)-epimethylenomycin A (**2**): 1.44 (s, 3 H), 1.68 (s, 3 H) 3.77 (m, 1 H) 5.81 (d, J = 2.6 Hz, 1 H), 6.41 (d, J = 2.6 Hz, 1 H)

smoothly and efficiently afforded the desired terminal olefinic esters when treated with O_3 at −78 °C in methanol followed by thermolysis at the reflux point of chloroform containing a small amount of pyridine.[46] Presumably, the pyridine reacts with the phenylselenenic acid, thereby eliminating the secondary reaction of this species with the newly generated terminal olefin.[47]

During the course of this venture into organoselenium chemistry we also examined the reaction of phenylselenolate with monoactivated cyclopropanes.[48,49] Previous to this investigation, with one exception,[50] nucleophilic cleavage of monoactivated cyclopropanes had been observed only in highly strained bicyclic systems.[51] Suffice it to say here that the cyclopropyl ring, monoactivated with either a ketone or nitrile functionality, suffers in good to excellent yield nucleophilic cleavage when subjected to this powerful anion; the two examples shown below are sufficient to illustrate this reaction. Best results with cyclopropyl ketones were obtained with sodium phenylselenolate, while nucleophile cleavage of cyclopropyl nitriles proceeds better with lithium phenylselenolate.



32 33

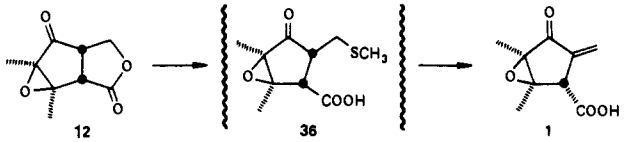


34 35

Unfortunately, all attempts to adapt the above organoselenium methodology to open the γ-lactone in cyclomethylenomycin A were singularly unsuccessful; only recovery of starting material or complex product mixtures were obtained under a wide variety of conditions. It appeared that a much milder method was required to effect the desired transformation.

(vii) **A Return to Sulfur Nucleophiles: Completion of the Stereospecific Total Synthesis of (±)-Methylenomycin A and Its Epimer.** Contemporary with our study of organoselenium reagents, Kelly, Dali, and Tsang[52] demonstrated that lithium thiomethoxide, a stable, nonhygroscopic solid, was an excellent and extremely mild reagent for alkyl–oxygen cleavage of esters and lactones. In particular, simple lactones in the presence of HMPA were found to undergo near-quantitative conversion to the corresponding ω-thiomethylcarboxylic acid at room temperature in only a few hours.

Armed with this information, we subjected cyclomethylenomycin A (**12**) to lithium thiomethoxide (2.0 equiv) in HMPA at room temperature for 4 h. To our surprise, the expected thioether (**36**) was not obtained; instead, crystalline

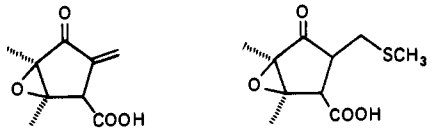


12 36 1

(±)-methylenomycin A, mp 88.5–89.0 °C, was isolated in 68% yield. Interestingly, sublimation of this material afforded a second crystalline form of (±)-methylenomycin A which melted at 107.5–108.5 °C.

That both materials were indeed (±)-methylenomycin A was apparent from its spectroscopic properties (i.e., IR, 60- and 220-MHz NMR, UV, and MS) as well as by direct comparison with an authentic sample.[53] Presumably the final conversion (**12** → **1**) proceeds via an initial nucleophilic cleavage of the lactone ring, followed in a subsequent step by facile base-induced (i.e., CH_3S^-) elimination of methanethiol. Support for this reaction pathway arises from the observation that treatment of **12** with only 1 equiv of lithium thiomethoxide yielded a mixture of **1** and **12**, while more than 1 equiv resulted in complete conversion.

Hoping to exploit this facile process for the elaboration of epimethylenomycin A, **13** was treated in a similar fashion; in this case only destruction of starting material resulted. However, by reducing the reaction time to exactly 110 min, a mixture of two compounds could be isolated. Examination of the high-field NMR spectrum of this mixture revealed resonances at δ 5.81 and 6.41, characteristic of the exomethylene group of the desired epimethylenomycin A (**2**); the second component was presumed by presence of a doublet at δ 2.15 to be the intermediate α-thiomethyl ether (**37**). Careful



2 37

chromatography on silica gel, elution first with chloroform and then with benzene–methanol (9:1), afforded an analytical sample of (±)-epimethylenomycin A (**2**) as an oil. For comparison purposes, we present in Table II the 220-MHz NMR data for (±)-epimethylenomycin A and (±)-methylenomycin A.

In summary, the stereospecific total synthesis of both (±)-methylenomycin A and its epimer, (±)-epimethylenomycin A (**2**), has been achieved. The synthetic route is both short and highly efficient (i.e., proceeds in 12 and 4% overall yield, respectively), affords both from a common intermediate (**14**) readily available from maleic anhydride and 2-pentyne through the agency of a photochemical [2 + 2] cycloaddition, and was effected without resort to protecting-group methodology and in the case of (±)-methylenomycin A (**1**) without aid of chromatographic separation. Such characteristics of a total synthesis are, and will continue to be, the guiding principles of research in our laboratory.

Experimental Section

Materials and Methods. Vapor phase chromatography was performed on an Aerograph Model 920 gas chromatograph employing one of the following columns: A, 12.5% OC-101, 10 ft × 0.25 in.; B, 6% Carbowax 1500, 10 ft × 0.25 in.; C, 6% SE-30, 10 ft × 0.25 in.; D, 6% QF-1, 10 ft × 0.25 in.; E, 25% QF-1, 10 ft × 0.25 in.; F, 1.5% OV-101, 10 ft × 0.25 in. The helium carrier gas flow rate was 100–120 mL/min and the oven temperature ranged from 160 to 190 °C. Compounds isolated by preparative VPC were obtained as either colorless oils or white solids. Melting points were taken on a Thomas-Hoover capillary melting apparatus and are corrected. Boiling points are uncorrected. Solutions were dried over $MgSO_4$ unless specified otherwise. IR and proton spectra were obtained for CCl_4, $CDCl_3$, or acetone-d_6 solutions, the former on a Perkin-Elmer Model 337 spectrophotometer and the latter on either a Varian A-60A (60 MHz), a HR 220 (220 MHz), or a Bruker WH 360 (360 MHz) spectrometer. ^{13}C NMR spectra were obtained in $CDCl_3$ on a JEOL PS-100 spectrometer. The internal standard for both ^1H and ^{13}C NMR experiments was Me_4Si. Low- and high-resolution mass spectra were obtained at the University of Pennsylvania Mass Spectrometry Service Center on a Hitachi Perkin-Elmer RMH-2 high-resolution

double-focusing electron impact spectrometer interfaced with a Kratos DS-50-S data system. Photochemical experiments were carried out with a Hanovia Model L mercury lamp (no. 679A-36) in a quartz immersion well using Corex 7740 as filter.

Preparation of Cyclobutene (15). A mixture of 5.98 g (61 mmol) of maleic anhydride, 5.19 g (76.2 mmol) of 2-pentyne, and 1.30 g (7.1 mmol) of benzophenone in 250 mL of acetonitrile was irradiated for 14 h. Removal of solvent and excess 2-pentyne in vacuo followed by vacuum distillation (bp 115 °C, 1.0 mm) afforded 7.92 g of **15** (79%). An analytical sample of **15** was obtained by preparative VPC on column A: IR 2975 (w), 1855 (w), 1777 (s), 1230 (w), 1070 (w), 970 (w), 805 cm^{-1} (s); NMR (CCl_4, 220 MHz) δ 1.11 (t, J = 8 Hz, 3 H), 1.84 (s, 3 H), 2.1-2.3 (br m, 2 H), 3.66 (t, J = 3 Hz, 1 H), 3.74 (t, J = 3 Hz, 1 H).

Anal. ($C_9H_{10}O_3$).

Preparation of Cyclobutenediol (16). To a magnetically stirred suspension of $LiAlH_4$ (3.92 g, 102 mmol) in 100 mL of anhydrous tetrahydrofuran was added dropwise a solution of anhydride **15** (5.23 g 31.4 mmol) in 60 mL of tetrahydrofuran. After refluxing for 40 h the excess hydride was destroyed by slow addition with cooling, 4 mL of water, 4 mL of 15% NaOH, and 12.0 mL of water. The aluminum salts were removed by filtration and the ether layer was dried over magnesium sulfate. Removal of solvents in vacuo gave 4.64 g (94%) of diol **16.** An analytical sample of **16** was obtained by preparative VPC on column A: IR 3300 (br, s), 2970 (s), 2880 (s), 1445 (w), 1038 cm^{-1} (w); NMR (CCl_4, 220 MHz) δ 0.99 (t, J = 8 Hz, 3 H), 1.57 (s, 3 H), 1.67-2.22 (comp br, 2 H), 2.75-3.03 (br, m, 2 H), 3.76 (m, 4 H), 4.55 (br, s, 2 H, D_2O exchange).

Anal. ($C_9H_{16}O_2$).

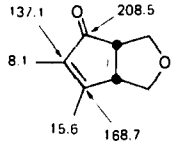
Preparation of Tetrahydrofuran (17). To a cold (0 °C) solution of 6.91 g of diol **16** (44.3 mmol) in 100 mL of dry pyridine was added a cold (10 °C) solution of 9.27 g (49.7 mmol, 1.2 equiv) of *p*-toluenesulfonyl chloride in 100 mL of dry pyridine. After standing for 18 h at 0 °C the mixture was warmed to room temperature and refluxed for 2 h under nitrogen. Workup consisted of extraction into ether and washing with 10% HCl. Removal of solvent in vacuo followed by distillation (bp 75 °C, 20 Torr) afforded 4.65 g of **17** (86%). An analytical sample of ether **17** was obtained by preparative VPC on column A: IR 2955 (s), 2940 (s), 1455 (w), 1070 (s), 1019 (w), 902 cm^{-1} (w); NMR (CCl_4, 220 MHz) δ 1.03 (t, J = 8 Hz, 3 H), 1.60 (s, 3 H), 1.86-2.17 (m, 2 H), 2.90-3.15 (comp m, 4 H), 3.58 (d, J = 9 Hz, 2 H).

Anal. ($C_9H_{14}O$).

Preparation of Diketone 18. A solution of 6.80 g (55.7 mmol) of tetrahydrofuran (**17**) in 70 mL of dry methanol was ozonized at −78 °C until the deep blue color of ozone persisted. The excess ozone was then removed by passing O_2 through the solution. To this solution was added 16 g (1.1 equiv) of triphenylphosphine at −78 °C and the mixture was allowed to warm to room temperature and stirred for an additional 2 h. Removal of solvents in vacuo and short-path distillation (bp 110 °C, 2 Torr) afforded 7.11 g (75%) of diketone **18.** An analytical sample of this compound was obtained by preparative VPC from column A: IR 2975 (w), 2940 (w), 2866 (w), 1710 (s), 1355 (w), 1175 (w), 1070 (w), and 930 cm^{-1} (w); NMR (CCl_4, 220 MHz) δ 1.05 (t, J = 8 Hz, 3 H), 2.14 (s, 3H), 2.42 (q, J = 8 Hz, 2 H), 3.43-3.57 (m, 2 H), 3.62-4.20 (m, 4 H).

Anal. ($C_9H_{14}O_3$).

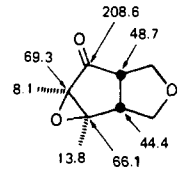
Preparation of Bicyclic Enone 14. A solution containing 7.11 g (42.4 mmol) of diketone **18** and 75 mL of 2% (v/w) methanolic sodium hydroxide (95% MeOH) was heated at reflux for 35 min. After cooling to room temperature the solution was carefully neutralized with 10% aqueous hydrochloric acid, extracted with ether, washed with brine, and dried over $MgSO_4$. Removal of solvents in vacuo gave 5.52 g (85%) of enone **14.** An analytical sample of this enone was obtained by preparative VPC on column B (mp 75 °C): IR 2970 (w), 2925 (w), 2860 (s), 1705 (s), 1655 (s), 1380 (w), 1203 (w), 1080 (s), 1065 (s), and 918 cm^{-1} (s); NMR (CCl_4, 220 MHz) δ 1.66 (s, 3 H), 2.03 (s, 3 H), 2.77 (t, J = 7 Hz, 1 H), 3.15 (t, J = 7 Hz, 1 H), 3.45-3.97 (comp m, 4 H). The natural abundance ^{13}C NMR spectrum of the enone **14** is summarized in the following structure.



8ridgehead carbons, 47.8 and 48.8 ppm
Furan CH_2O carbons, 68.5 and 70.1 ppm

Anal. ($C_9H_{12}O_2$).

Preparation of Epoxy Ketone 19. To a solution of 2.96 g (19.5 mmol) of enone **14** in 80 mL of methanol were added 2.20 mL of 3 N NaOH (6.6 mmol) and 8.0 mL of 30% hydrogen peroxide. This solution was kept at −20 °C with addition of aliquots of 3.0 N NaOH (2.20 mL) being added at 4 and 8 h. After 21 h the reaction was terminated by pouring the mixture into brine and extracting with 150 mL of ether. After drying over $MgSO_4$, removal of solvent in vacuo afforded 2.95 g (90%) of an oily epoxy ketone **19.** An analytical sample of this compound was obtained by preparative VPC on column C: IR 2975 (m), 2940 (m), 2865 (s), 1745 (s), 1380 (w), 1095 (s), 1080 (s), 1055 (m), and 917 cm^{-1} (s); NMR (CCl_4, 220 MHz) δ 1.35 (s, 3 H), 1.50 (s, 3 H), 2.74 (t, J = 7 Hz, 1 H), 3.05 (d, t, J = 4, 8 Hz, 1 H), 3.80-3.82 (m, 3 H), 4.53 (d, J = 10 Hz, 1 H). The natural abundance ^{13}C NMR spectrum of the epoxy ketone **19** is summarized in the following structure.



Furan CH_2O carbons, 68.7 and 71.2 ppm

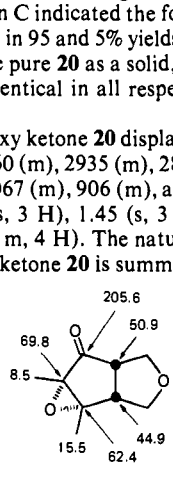
Anal. ($C_9H_{12}O_3$).

Formation of Allylic Alcohol 21. A magnetically stirred solution of 974 mg (6.40 mmol) of enone **14** in 15 mL of benzene was treated dropwise with 1.5 equiv of diisobutylaluminum hydride (5.12 mL, 2 M in toluene) at 0 °C. After 2 h at 0 °C the reaction mixture was worked up by addition of 20 mL of methanol and stirring for 1 h at 5 °C. Filtration of the resultant salts and removal of solvent in vacuo gave 1.01 g of allylic alcohol **21** (94%). An analytical sample of alcohol **21** was obtained by preparative VPC on column D: IR 3410 (s), 2905 (s), 2440 (s), 1445 (m), 1395 (m), 1118 (m), 1060 (w), 1010 (m), 970 (m), 905 cm^{-1} (s); NMR (CCl_4, 220 MHz) δ 1.62 (s, 6 H), 2.76 (m, 1 H), 2.96 (t, J = 7 Hz, 1 H), 3.23-3.37 (m, 2 H), 3.48-3.75 (m, 2 H, 1 H, D_2O exchange), 4.15 (d, J = 9 Hz, 1 H), 4.31 (d, J = 8 Hz, 1 H).

Anal. ($C_9H_{14}O_2$).

Preparation of Epoxy Ketone 20. To a stirred solution containing 2.17 g of alcohol **21** (14.1 mmol) in 95 mL of methylene chloride, cooled in an ice bath, was added dropwise 2.95 g (1.2 equiv, 16.9 mmol) of *m*-chloroperoxybenzoic acid in 45 mL of methylene chloride. This solution was maintained at 0 °C for 2 h and then filtered to remove precipitated *m*-chlorobenzoic acid. After removal of solvent in vacuo, purification of the resultant oil by silica gel column chromatography using ether as eluent gave 1.98 g of an epoxy alcohol (87%). This material (12.2 mmol) was dissolved in 65 mL of anhydrous acetone and treated at 0 °C with 5.0 mL (1.1 equiv) of Jones reagent (2.7 M). Excess Jones reagent was destroyed by addition of 2-propanol. After extraction into ether and drying over $MgSO_4$, removal of solvent in vacuo gave 1.80 g (88%) of an oil. Analysis of VPC on column C indicated the formation of a major and minor epoxide (**20** and **19** in 95 and 5% yields respectively). Preparative VPC on column C gave pure **20** as a solid, mp 61-62 °C, and **19** as an oil. The latter was identical in all respects with epoxy ketone **19** prepared previously.

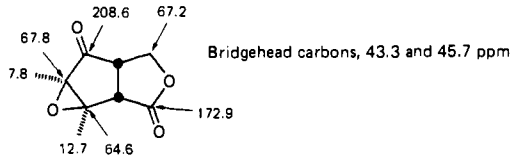
Epoxy ketone **20** displayed the following spectroscopic properties: IR 2960 (m), 2935 (m), 2865 (m), 1740 (s), 1455 (w), 1195 (w), 1097 (2), 1067 (m), 906 (m), and 895 cm^{-1} (w); NMR (CCl_4, 220 MHz) 1.32 (s, 3 H), 1.45 (s, 3 H), 2.96-2.95 (comp m, 2 H), 3.52-4.05 (comp m, 4 H). The natural abundance ^{13}C NMR spectrum of the epoxy ketone **20** is summarized in the following structure.



Furan CH_2O carbons, 68.6 and 69.5 ppm

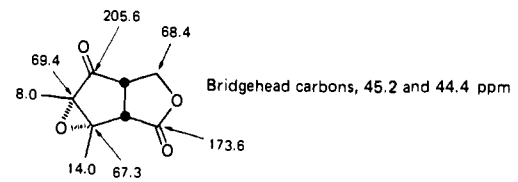
Preparation of Cyclomethylenomycin A (12). A mixture consisting of 10 g of sodium periodate in 200 mL of water and 300 mg of RuO_2 in 200 mL of carbon tetrachloride was vigorously stirred until generation of the deep yellow color of ruthenium tetroxide. A solution containing 2.62 g (15.6 mmol) of epoxy ketone **19** in 100 mL of carbon tetrachloride was added dropwise to the above two-phase system of

ruthenium tetroxide. The deep yellow color faded immediately but returned after several hours. Stirring was continued for an additional 5 days at room temperature. After this period of time the phases were separated and 500 μL of 2-propanol was added to the CCl_4 layer to precipitate RuO_2. After the mixture was dried over magnesium sulfate, removal of solvents in vacuo gave 1.31 g (46%) of pale white, crystalline lactone **12**. Recrystallization from petroleum ether (30–60 °C) gave an analytical sample (mp 80.5–81.0 °C): IR 3025 (m), 2980 (w), 1775 (s) 1750 (s), 1380 (m), 1180 (s) 1118 (w), 1085 (m), 1070 (s), 1028 (s), 900 (m), and 800 cm^{-1} (s); NMR (CCl_4, 220 MHz) δ 1.46 (s, 3 H), 1.65 (s, 3 H), 3.17 (t, J = 9 Hz, 1 H), 3.59 (d, J = 9 Hz, 1 H), 4.42 (t, J = 10 Hz, 1 H), 4.57 (d, J = 10 Hz, 1 H); mass spectrum m/e 182.0564 (M^+, calcd for $C_9H_{10}O_4$, 182.0576). The natural abundance ^{13}C NMR spectrum of keto lactone **12** is summarized in the following structure.



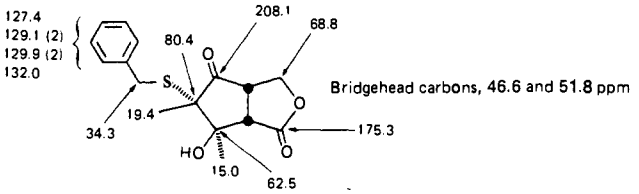
Anal. ($C_9H_{10}O_4$).

Preparation of Epoxy Keto Lactone (Epicyclomethylenomycin A) (13). Following an identical procedure as outlined for the preparation of lactone **12**, compound **13** (45%, mp 106.5–107.0 °C) from petroleum ether (30–60 °C) was prepared from **20**: IR 2930 (w), 1775 (s), 1750 (s), 1480 (m), 1192 (s), 1120 (w), 1100 (m), 1052 (m), 1040 (s), 882 cm^{-1} (w); NMR ($CDCl_3$, 220 MHz) δ 1.43 (s, 3 H), 1.72 (s, 3 H), 3.13–3.39 (complex m, 2 H), 4.22 (d, d, J = 4, 12 Hz, 1 H), 4.46 (d, J = 10 Hz, 1 H). The natural abundance ^{13}C NMR spectrum of the epoxy keto lactone **13** is summarized in the following structure.



Anal. ($C_9H_{10}O_4$).

Preparation of Benzylthiol Adduct 30. A solution consisting of 48.3 mg of lactone **12** (0.265 mmol), 49.4 mg (0.397 mmol, 1.5 equiv) of benzenethiol, and 98.6 mg of diazobicyclononene (DBN) in 5 mL of dry benzene was allowed to stir overnight at room temperature. The solution was next poured into 50 mL of ether, washed with 10% HCl, and dried over $MgSO_4$. Removal of solvent in vacuo gave 92.9 mg of crystalline thioether **30**. Recrystallization from benzene furnished an analytical sample (mp 188.5–189.5 °C): IR 3595 (m), 2915 (w), 1767 (s), 1738 (s), 1170 (m), 1052 (m), 1019 (w), 900 cm^{-1} (w); NMR (acetone-d_6, 220 MHz) δ 1.45 (s, 3 H), 1.57 (s, 3 H), benzylic AB [δ 3.19 (d, J = 12 Hz, 1 H), 3.75 (d, J - 12 Hz, 1 H)], 3.47 (t, J = 10 Hz, 1 H), 3.50 (d, J = 12 Hz, 1 H), 4.32 (d, d, J = 2, 12 Hz, 1 H), 4.57 (t, J = 10 Hz, 1 H), 5.01 (s, br, 1 H, D_2O exchange), 7.25 (s, 5 H). The natural abundance ^{13}C NMR spectrum (acetone-d_6) of the benzylthiol adduct **30** is summarized in the following structure.

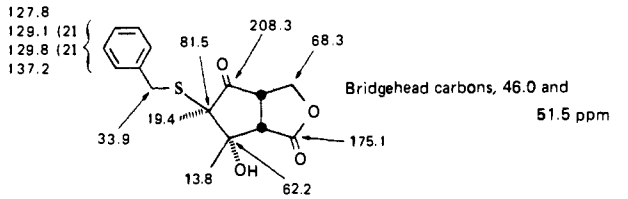


Anal. ($C_{16}H_{18}O_4S$).

Preparation of (±)-Methylenomycin A (1). A solution of 50 mg (0.275 mmol) of lactone **12** in 1 mL of freshly distilled hexamethylphosphoric triamide (HMPA) was treated under a nitrogen atmosphere with 40 mg of lithium thiomethoxide dissolved in 1 mL of HMPA. After 4 h the reaction mixture was treated with 5 mL of H_2O and then added to ether. After separation of the organic phase, the aqueous phase was carefully acidified to pH 6 with cold 10% HCl and then extracted with ether and dried. Removal of the solvent in vacuo afforded a pale yellow oil, which after filtration through a short plug of silica gel with ether and removal of the solvent in vacuo gave 34.2 mg of crystalline **1** (mp 88.5–89.0 °C) which after sublimation (70–75 °C, 0.025 mmHg) melted at 107.5–108 °C. Both crystalline forms possessed spectral properties (IR, 60- and 220-MHz NMR, and MS) which were identical in all respects with those derived from an authentic sample.[53]

Preparation of (±)-Epimethylenomycin A (2). A solution containing 40 mg (0.220 mmol) of lactone **13** in 1 mL of freshly distilled HMPA was added to a solution of 35 mg of lithium thiomethoxide in 1 mL of HMPA and the mixture stirred under nitrogen for 1.75 h. At this point the reaction mixture was poured into ether, washed with 10% aqueous HCl and brine, and dried over $MgSO_4$. Removal of solvent in vacuo afforded 28.5 mg of yellow oil which consisted mainly of (±)-epimethylenomycin A (**2**). An analytical sample of (±)-epimethylenomycin A was obtained by chromatography on silica gel. Development first with chloroform and then with benzene–methanol (90:10) gave 12.5 mg of a pale yellow, viscous oil: R_f 0.30; IR 3500–2550 (br), 1740 (s), 1715 (s), 1695 (m), 1042 (m), 970 cm^{-1} (m); NMR ($CDCl_3$, 220 MHz) δ 1.44 (s, 3 H), 1.68 (s, 3 H), 3.77 (m, 1 H), 5.81 (d, J = 2.6 Hz, 1 H), 6.41 (d, J = 2.6 Hz, 1 H); mass spectrum m/e 182.0573 (M^+, calcd for $C_9H_{10}O_4$, 182.0576).

Formation of Benzylthiol Adduct 31. A solution consisting of 50.6 mg (0.278 mmol) of epicyclomethylenomycin A (**13**), 49 μL (0.417 mmol, 1.5 equiv) of benzylthiol, and 69 μL (0.556 mmol, 2.0 equiv) of diazabicyclononene (DBN) in 5 mL of dry benzene was allowed to stir for 5 h at room temperature, after which TLC analysis showed the reaction to be complete. The solution was extracted into 50 mL of ether, washed with 10% HCl solution and brine, and dried over magnesium sulfate. Removal of solvent in vacuo and recrystallization from benzene afforded 44 mg (52%) of a white, crystalline adduct (**31**). An analytical sample (mp 154.5–155.5 °C) had IR 3610 (m), 2990 (m), 1767 (s), 1739 (s), 1385 (s), 1195 (s), 1080 (m), 1037 (s), 692 cm^{-1} (m); NMR (acetone-d_6, 220 MHz) δ 1.42 (s, 3 H), 1.50 (s, 3 H), 3.32 (d, J = 12 Hz, 1 H), benzylic AB [3.48 (d, J = 12 Hz, 1 H), 3.70 (d, J = 12 Hz, 1 H)], 3.88 (m, 1 H), 4.04 (m, 1 H), 4.50 (d, d, J = 2, 12 Hz, 1 H), 5.22 (br s, 1 H, D_2O exchange), 7.25 (s, 5 H). The natural abundance ^{13}C NMR spectrum (acetone-d_6) of the benzylthiol adduct **31** is summarized in the following structure.



Anal. ($C_{16}H_{18}O_4S$).

Preparation of Lactone 25. A mixture consisting of 2.0 g of sodium periodate in 50 mL of carbon tetrachloride and 50 mL of water was vigorously stirred until generation of the yellow color of ruthenium tetroxide. A solution containing 238 mg (1.83 mmol) of tetrahydrofuran ester **22** in 5 mL of carbon tetrachloride was added. Stirring was continued for a period of 40 h. After the layers were separated, 2-propanol was added to precipitate ruthenium dioxide in the carbon tetrachloride layer. Removal of solvent in vacuo gave 105.5 mg (40%) of a colorless oil. An analytical sample of this compound, obtained on column A, was identical in all respects (VPC, IR, 220- and 60-MHz NMR) with an authentic sample of **25** prepared according to published procedures.[54]

Preparation of Lactones 26 and 27. The oxidation procedure employed was identical with that used in the preparation of lactone **25**. The reaction mixture was separated by preparative VPC on column E to afford lactone **26** (46%), IR and NMR data identical with those published[55] for this compound, and lactone **27** (30%), identical with an authentic sample of this compound prepared according to the published procedure.[56]

General Procedure for Preparation of Phenylselenide Esters. A solution of 312 mg (1.0 mmol) of diphenyl diselenide in 5 mL of dry dimethylformamide was deoxygenated by bubbling nitrogen through for 20 min. Sodium borohydride, 85 mg (2.25 mmol), was added (vigorous evolution of H_2) and the temperature slowly raised in an oil bath to 100 °C under nitrogen. The lactones were introduced (1.85 mmol) as solutions in 1 mL of dimethylformamide and the oil-bath temperature was raised to 120 °C and kept at this temperature for the indicated times (Table I). The reaction mixtures were cooled, extracted into 100 mL of ether, washed with 10% HCl and brine, and dried over $MgSO_4$. Removal of solvents in vacuo gave the acids which

were esterified by treatment with excess ethereal diazomethane. Analytical samples of these compounds were obtained by preparative VPC on column F.

Methyl 4-Phenylselenylbutyrate: IR 3060 (m), 2950 (s), 1735 (s), 1435 (s), 1205 (s), 902 (s), 685 cm^{-1} (s); NMR (CCl_4, 220 MHz) δ 2.00 (m, 2 H), 2.45 (t, J = 7.0 Hz, 2 H), 2.93 (t, J = 7.0 Hz, 2 H), 3.65 (s, 3 H), 7.27 (m, 3 H), 7.50 (m, 2 H).

Anal. ($C_{11}H_{14}O_2Se$).

Methyl 5-Phenylselenylpentanoate: IR 2950 (m), 1735 (s), 1440 (m), 1205 (s), 1025 (w), 687 cm^{-1} (m); NMR (CCl_4, 220 MHz) δ 1.72 (m, 4 H), 2.25 (t, J = 7.0 Hz, 2 H), 2.86 (t, J = 7.0 Hz, 2 H), 3.61 (s, 3 H), 7.20 (m, 3 H), 7.43 (m, 2 H).

Anal. ($C_{12}H_{16}O_2Se$).

Methyl 6-Phenylselenylhexanoate: IR 3070 (w), 2945 (m), 1725 (s), 1580 (m), 1145 (m), 1027 (m), 692 cm^{-1} (m); NMR (CCl_4, 220 MHz) δ 1.43 (m, 2 H), 1.45–1.77 (comp m, 4 H), 2.23 (t, J = 7.0 Hz, 2 H), 2.84 (t, J - 7.0 Hz, 2 H), 3.61 (s, 3 H), 7.18 (m, 3 H), 7.41 (m, 2 H).

Anal. ($C_{13}H_{18}O_2Se$).

Methyl 7-Phenylselenylhepanoate: IR 3060 (w), 2925 (s), 1735 (s), 1440 (m), 1180 (s), 1025 (w), 695 cm^{-1} (m); NMR (CCl_4, 220 MHz) δ 1.27–1.77 (m, 8 H), 2.22 (t, J = 7.0 Hz, 2 H), 2.84 (t, J = 7.0 Hz, 2 H), 3.60 (s, 3 H), 7.18 (m, 3 H), 7.45 (m, 2 H).

Anal. ($C_{14}H_{20}O_2Se$).

Methyl 12-Phenylselenyldodecanoate: IR 2940 (m), 2855 (w), 1738 (s), 1180 (m), 690 cm^{-1} (m); NMR (CCl_4, 220 MHz) δ 1.27 (br, s, 14 H), 1.52–1.75 (m, 4 H), 2.20 (t, J = 7.0 Hz, 2 H), 2.82 (t, J = 7.0 Hz, 2 H), 3.59 (s, 3 H), 7.17 (m, 3 H), 7.40 (m, 2 H).

Anal. ($C_{19}H_{30}O_2Se$).

5-Phenylselenyl-2-butanone: IR 3060 (w), 2950 (m), 1714 (s), 1360 (m), 1180 (m), 1035 (m), 699 cm^{-1} (m); NMR (CCl_4, 220 MHz) δ 1.90 (m, 2 H), 2.01 (s, 3 H), 2.50 (t, J = 7.0 Hz, 2 H), 2.87 (t J = 7.0 Hz, 2 H), 7.20 (m, 3 H), 7.45 (m, 2 H).

Anal. ($C_{11}H_{14}OSe$).

Methyl 4-Phenylselenylpentanoate: IR 3075 (w), 2960 (m), 1727 (s), 1580 (w), 1445 (s), 1180 (s), 1028 (m), 694 cm^{-1} (m); NMR (CCl_4, 220 MHz) δ 1.43 (d, J = 7.5 Hz, 3 H), 1.86 (q, J = 7.0, 7.0 Hz, 2 H), 2.66 (t, J = 7.0 Hz, 2 H), 3.22 (q, J = 7.0, 7.0 Hz, 1 H), 3.61 (s, 3 H), 7.21 (m, 3 H), 7.50 (m, 2 H).

Anal. ($C_{12}H_{16}O_2Se$).

General Procedure for Preparation of ω-Olefinic Esters from Selenides. The selenide esters were oxidized to the corresponding selenoxides with ozone in methylene chloride. To this end, ozone was introduced into methylene chloride solutions of the respective ester at −78 °C until a blue color (excess ozone) persisted; the excess ozone was then removed by passing O_2 through the solution. Next the CH_2Cl_2 was removed through a Vigreux column and chloroform added which contained several drops of pyridine to scavenge phenylselenenic acid. The eliminative process was effected by heating the reaction mixture at reflux for several hours. Removal of the solvent in vacuo and distillation of the resultant liquids afforded the respective olefinic ester (see Table I).

Methyl 3-Butenoate: 70% yield; NMR (CCl_4, 220 MHz) δ 3.03 (d, J = 7.5 Hz, 2 H), 3.63 (s, 3 H), 5.11 (m, 2 H), 5.88 (comp m, 1 H). This material was identical with a sample prepared by diazomethane esterification of vinylacetic acid.

Methyl 4-Pentenoate: 65% yield; NMR (CCl_4, 60 MHz) δ 2.33 (s, 2 H), 2.39 (s, 2 H), 3.55 (s, 3 H), 4.92 (m, 1 H), 5.16 (m, 1 H), 5.91 (m, 1 H). This material had NMR data in accord with the published NMR for this compound.[57]

Methyl 5-Hexenoate: 71% yield; IR 3090 (w), 2950 (m), 1735 (s), 1640 (w), 1178 (s), 920 cm^{-1} (s); NMR (CCl_4, 60 MHz) δ 1.48–2.43 (comp m, 6 H), 3.60 (s, 3 H), 4.83 (m, 1 H), 5.06 (m, 1 H), 5.71 (m, 1 H).

Methyl 6-Heptenoate: 70.5% yield; IR 3080 (m), 2940 (s), 1737 (s), 1640 (m), 1440 (s), 1180 (s), 992 (m), 916 cm^{-1} (s); NMR (CCl_4, 60 MHz) δ 1.35–1.81 (m, 4 H), 1.86–2.41 (m, 4 H), 3.60 (s, 3 H), 4.86 (m, 1 H), 5.08 (m, 1 H), 5.63 (m, 1 H).

Methyl 11-Dodecenoate: 60.5% yield; this material was identical with a sample prepared by a photochemical Arndt–Eistert chain homologation of 10-undecenoic acid.

Methyl *trans*-3-Pentenoate: 33% yield; IR 970 cm^{-1} (s) trans double bond; NMR (CCl_4, 220 MHz) δ 1.68 (br, s, 3 H), 2.91 (br, s, 2 H), 3.60 (s, 3 H), 5.40 (m, 2 H). This material has NMR data in accord with published NNR data for this compound.[58]

Methyl *cis*-3-Pentenoate: 11% yield; NMR (CCl_4, 220 MHz) δ 1.68 (br, s, 3 H), 2.91 (br, s, 2 H), 3.60 (s, 3 H), 5.40 (m, 2 H). This material has NMR data in accord with published NMR data for this compound.[58]

Arndt–Eistert Chain Homologation of 10-Undecenoic Acid.[59] A solution containing 247.4 mg (1.34 mmol) of 10-undecenoic acid in 2 mL of benzene was treated with 194 mg (1.15 equiv) of oxalyl chloride for 3 h at room temperature. Distillation (Kugelrohr) of the residue after removal in in vacuo of the benzene and excess oxalyl chloride afforded 244 mg (90%) of the corresponding acid chloride [IR 2935 (s), 1800 (s), 912 cm^{-1} (m)]. The acid chloride was then dissolved in 20 mL of ether and added dropwise with stirring to an ethereal solution of CH_2N_2 (3.5 equiv); 239 mg (95%) of the corresponding diazo ketone [IR 2935 (s), 2100 (s), 1645 (m), 910 cm^{-1} (m)] was isolated. The diazo ketone was dissolved in 70 mL of MeOH and irradiated through a Pyrex filter for 60 min. The photosylate was poured into 50 mL of H_2O and extracted with ether and the combined organic phase was washed with H_2O and brine and dried. Removal of solvent in vacuo gave 205 mg (86%) of the chain homologated ester. An analytical sample of this compound was obtained by preparative VPC on column D: IR 2920 (s), 1737 (s), 917 cm^{-1} (m); NMR (CCl_4, 220 MHz) δ 1.29 (br, s, 12 H), 1.59 (m, 2 H), 2.04 (m, 2 H), 2.23 (t, J = 7.0 Hz, 2 H), 3.64 (s, 3 H), 4.95 (m, 2 H), 5.77 (m, 1 H).

Preparation of Cyclohexanone (33). A solution containing 470 mg (1.5 mmol) of diphenyl diselenide, 5 mL of tetrahydrofuran, and 1.5 mL of 50% aqueous hypophorphorous acid was heated at reflux for 20 min. Upon cooling the resultant phenylselenol was extracted into 40 mL of benzene. The benzene solution was vacuum filtered through a pad of magnesium sulfate and transferred to a dry reaction vessel fitted with condenser and nitrogen inlet. Lithium phenylselenolate was generated via addition of 1.05 equiv of *n*-butyllithium. After 10 min at room temperature the reaction vessel was charged with 330.5 mg (3.0 mmol) of cyclopropyl ketone (**32**) and 0.055 mmol of 12-crown-4. This solution was heated at reflux for 15 h and cooled. The reaction was quenched by addition of 10% HCl solution, the mixture extracted with ether, and the organic material washed with water and brine and dried. Removal of the solvent in vacuo afforded 841.6 mg of ketone **33.** A portion of this material was chromatographed on a 500-μ silica plate (developed with methylene chloride) to yield an analytical sample (64% yield) as a pale yellow oil: IR 3065 (w), 2940 (m), 1712 (s), 1230 (m), 1027 (w), 690 cm^{-1} (m); NMR (CCl_4, 220 MHz) δ 1.29–1.75 (m, 4 H), 2.22–2.50 (m, 5 H), 2.88 (m, 2 H), 7.20 (m, 3 H), 7.47 (m, 2 H).

Anal. ($C_{13}H_{16}OSe$).

3-Phenylselenylbutyronitrile (35). A solution of 312 mg (1.0 mmol) of diphenyl diselenide in 5 mL of dry dimethylformamide was degassed by bubbling nitrogen through the solution for 20 min. Sodium borohydride, 85 mg (2.25 mmol), was added and the temperature of the solution raised to 100 °C under nitrogen; 140 mg (2.0 mmol) of cyclopropyl cyanide (**34**) was then introduced and the temperature raised to 125 °C for a period of 12 h. After this period, the mixture was cooled and extracted with ether, and the organic material was washed with 10% HCl solution to remove dimethylformamide and with brine and dried. Removal of solvent in vacuo afforded 383 mg of nitrile **35. 35** (156 mg) was placed on a 1000-μ silica plate and developed with ether–pentane (50:50) to afford 84.5 mg (47% yield) of **35** as a colorless oil: IR 3075 (m), 2930 (m), 2245 (m), 1475 (m), 1435 (s), 1250 (s), 1020 (s), 696 cm^{-1} (s); NMR (CCl_4, 220 MHz) δ 1.97 (m, 2 H), 2.47 (t, J = 7.0 Hz, 2 H), 2.96 (t, J = 7.0 Hz, 2 H), 7.29 (m, 3 H), 7.47 (m, 2 H).

Anal. ($C_{10}H_{11}NSe$).

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i ii

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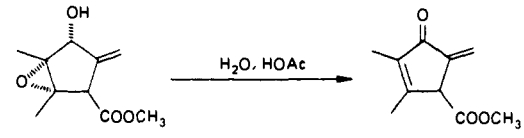

iii iv

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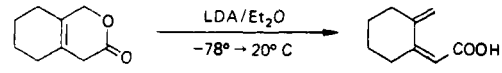

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Reduction of the Syn–Anti Glycosyl Conformational Barrier in 2'-Deoxyadenosine upon Binding to Ethidium Bromide. Evidence from Ultrasonic Relaxation Measurements[1]

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Abstract: The unimolecular relaxation found by ultrasonic relaxation in dilute aqueous solutions of 2'-deoxyadenosine was examined in the absence and presence of ethidium bromide (a model for an intercalating drug) and indole-3-acetic acid at pH 7.0 (a model for tryptophan as a potential binding site on a protein). This relaxation, which had previously been assigned to the syn-anti glycosyl isomerization, changes both in terms of f_r and amplitude in the presence of the added reagents. Both ethidium bromide and indole-3-acetic acid shift the relaxation frequency, f_r, to higher values. Detailed analysis of the data in the presence of varying amounts of ethidium bromide indicates that the apparent activation energy to syn-anti isomerization is decreased when 2'-deoxyadenosine is bound to ethidium bromide. ^{1}H NMR studies were performed to elucidate the mechanism of binding. Assuming a 1:1 2'-deoxyadenosine-ethidium bromide (heterostack) complex, ^{1}H NMR (in ^2H_2O) gives a $K_{\text{heterostack}}$ of ca. 300 M^{-1} compared to the value derived from the ultrasonic data (in H_2O) of ca. 400 M^{-1}.

Introduction

For several years now this laboratory has been engaged in developing the ultrasonic relaxation approach to study the kinetics and thermodynamics of the syn-anti glycosyl isomerization in nucleosides and nucleotides.[3-5] Based on our results as well as on those of Rhodes and Schimmel[6] it has been well established that adenosine,[3,6] 2'-deoxyadenosine,[6] and adenosine 3',5'-cyclic monophosphate[4] give rise to a unimolecular relaxation in the 10-100-MHz frequency range. With nucleotides the relaxation is masked by other fast processes.[7] We have also demonstrated that urea destacks aggregated nucleic bases[8] and that the stacking and syn-anti glycosyl conformational degree of freedom are coupled to each other.[4] While the method appears to be applicable primarily to purine nucleosides (in our hands a variety of pyrimidine nucleosides did not give rise to unimolecular relaxation in the frequency range 10-300 MHz accessible on our instrumentation), there are several aspects of the behavior of these molecules that are worthy of delineation and delineation of which can uniquely be performed by our technique.

One of the important questions concerning the solution behavior of nucleosides and nucleotides has to do with possible changes in syn-anti rotational barrier and equilibrium constant upon binding either to a receptor or to an intercalating drug. We have now examined that ultrasonic relaxation of 2'-deoxyadenosine attributable to the syn-anti isomerization in the presence of indole-3-acetic acid (a model for tryptophan as potential hydrophobic binding site on a protein) and ethidium bromide (as a model for an intercalating drug). Our choice of 2'-deoxyadenosine is dictated by the fact that it has relatively high solubility compared to other naturally occurring purine nucleosides[9] and the amplitude of the ultrasonic relaxation is large enough for this molecule to be readily quantitated. Herein we report our results which for the first time demonstrate that binding of 2'-deoxyadenosine to ethidium bromide or indol-3-acetic acid reduces the barrier to syn-anti rotations, i.e., increases f_r for the process. In addition, to confirm the ultrasonic results, we have determined the $K_{\text{association}}$ between ethidium bromide and 2'-deoxyadenosine by ^{1}H NMR, to our knowledge the first determination of this association constant between this intercalator and a nucleoside. The technique we have employed should be of general applicability.

Experimental Section

Materials. 2'-Deoxyadenosine and ethidium bromide were from Sigma; indole-3-acetic acid was from Eastman. All of these chemicals were used without further purification. Distilled, deionized, degassed water was employed for all solutions. pH was adjusted and measured with a Radiometer pH meter No. 26 equipped with glass and calomel electrodes.

Methods. Ultrasonic relaxation measurements were taken either on a pulse or a swept frequency resonator instrument. The measurements of the ultrasonic absorption were carried out at the odd harmonic frequencies of a 5-MHz X-cut quartz transducer by means of the pulse technique (MATEC Model 765 and 960). The frequency range was from 15 to 280 MHz.[10] A swept frequency resonator was also used for the absorption measurements in the frequency range of 12.5-38 MHz (the instrumentation employed is similar to Eggers' design[11]). The sound velocity was measured at 15 MHz. The ultrasonic cell was immersed in a water bath which was maintained within ±0.02°C.

^{1}H NMR measurements were performed on a JEOL PSFT-100 Fourier transform instrument at 28 °C (probe temperature). Chemical shifts were measured against 3-trimethylsilyl-*2,2,3,3-*$^2H_4$-propionic acid sodium salt (Stohler Isotope) in 99.8% ^2H_2O (Stohler Isotope). Typically 50-1000 transients of ca. 4 s repetition time and 45-60° pulse width were collected. For the most dilute samples the WEFT[12] technique was employed attempting to beat down the residual HO^2H signal by collecting transients with the $(180\text{-}\tau\text{-}90)T_1$ sequence where τ is the delay appropriate to null the HO^2H signal. On account of the very limited solubility of both ethidium bromide and 2'-deoxyadenosine, but the enhanced solubility of each in the presence of the other, several methods were attempted to prepare the samples. The method finally adopted involved preparing a stock solution of ethidium bromide (0.0025 M) and directly weighing in the 2'-deoxyadenosine into 5-mL volumes of this solution.

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