Tetrahedron Vol 29, pp 1465 to 1472 Pergamon Press 1973 Printed in Great Britain

STRUCTURE OF A NOVEL LIPID FROM THE ANTIBIOTIC DIUMYCIN[1,*]

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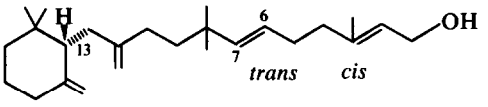
(*Received in the USA* 16 *November* 1972; *Received in the UK for publication* 22 *January* 1973)

Abstract—The structures of diumycinol (1), isodiumycinol (2), and diumycene (3), the nonisoprenoid C_{25} lipids obtained by acid hydrolysis of the antibiotic diumycin, have been determined by analysis of spectral data and the identification of a number of degradation products. Examination of the ORD of a degradation product gave the absolute configuration of the asymmetric center in these compounds.

Since 1965, several groups of non-toxic phosphorus-containing antibiotics have been reported that exhibit the unique and remarkable property of long duration of action *in vivo* against gram-positive bacteria.† Diumycin,[2a] for example, afforded 50% protection to mice when given subcutaneously, 6·7 mg/kg, 14 days prior to challenge with a lethal dose ($1000 \times LD_{50}$) of *Streptococcus pyrogenes* C_{203}. Similarly, moenomycin gave 100% protection to mice when given subcutaneously, 5 mg/kg, 10 days prior to infection. Although none of the structures of these substances has been fully elucidated, these antibiotics are known to be acidic compounds with molecular weight 1800 ± 100, and empirical formulas in the range $C_{65-75}H_{100-125}N_{5-7}O_{35-45}O$. Acid hydrolysis under a variety of conditions has yielded numerous degradation products, including, as in moenomycin A, glucosamine, quinovosamine, glucose, glycine, 4,5-dihydroxy-3-methylcyclopenten-2-one-1, 2-aminocyclopentanedione-1,3, and a C_{25} lipid.[3] Not all of these products, however, have been obtained from all of the moenomycins or from all of the other antibiotics studied. Structures have been assigned to the optically inactive C_{25} lipids obtained by acid hydrolysis of the related antibiotics moenomycin[4] and prasinomycin.[5] We now present our evidence for the structures of the optically active C_{25} lipids, diumycinol (1), isodiumycinol (2), and diumycene (3), derived from the diumycins.

Acid hydrolysis (1N HCl, 100°, 30 min), either of the individual diumycins or of the diumycin mixture, yields a chloroform-soluble oil that can be resolved by silica gel chromatography (column or preparative TLC) to give two alcohols, diumycinol and isodiumycinol, and a hydrocarbon mixture, diumycene.



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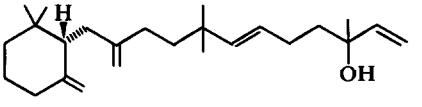

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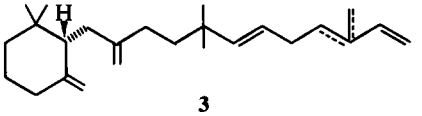

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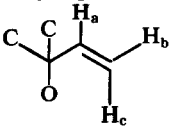
Isodiumycinol (2)

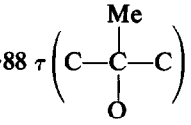
Molecular distillation of isodiumycinol, obtained by preparative TLC, gave a colorless oil having $[\alpha]_D^{26} + 5{\cdot}6°$ ($c = 1{\cdot}0$ g/100 ml EtOH). Elemental analysis and mass spectrometry, *m/e* 358 (M+), indicated that the substance had the formula $C_{25}H_{42}O$. The infrared spectrum (neat) of isodiumycinol showed absorptions at 3400 (OH), 1800–1600 (C=C) 1380 and 1360 (suggesting Me—C—Me), and 995, 975, 925, and 890 cm^{-1} (terminal methylene and terminal vinyl), whereas its ultraviolet spectrum (EtOH) showed no absorption above 205 nm, indicating the absence of conjugated double bonds. The PMR spectrum of isodiumycinol ($DCCl_3$, 60-MHz) allowed the proton assignments shown in Table 1.

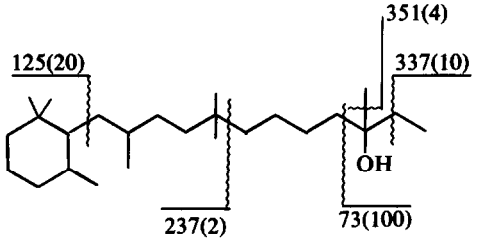
*Four components have been isolated, so far, from the diumycin mixture of antibiotics, including two 257-nm chromophore-containing components, diumycin A and diumycin A′, and two non-chromophore-containing components, diumycin B and diumycin B′; W. A. Slusarchyk, J. Bouchard-Ewing, and F. L. Weisenborn, manuscript in preparation.

†Members of this family of antibiotics include: diumycin,[2a] macarbomycin,[2b] moenomycin,[2c] prasinomycin,[2d] 8036RP,[3e] 11,837RP,[2f] and 19,402RP.[2g]

Table 1

Signal	Assignment
τ 9·14 (s, 3H) τ 9·07 (s, 3H) τ 9·03 (s, 6H)	C—CMe_2C C—CMe_2—C=C
τ 8·72 (s, 3H)	Me—C(=O)—C
τ 7·7–8·65 (m, ~ 18H)	Methylene protons
τ 5·20–5·55 (m, 3–4H)	2 C(H)=C(H)
τ 4·61 (m, 2H)	Vinylic protons
τ 4·96 (q, 1H, $J = 10, J = 2$)H_b τ 4·82 (q, 1H, $J = 17, J = 2$)H_c τ 4·05 (q, 1H, $J = 17, J = 10$)H_a	

Isodiumycinol, on reduction with Adams catalyst in either ethanol or acetic acid, consumed four equivalents of hydrogen, yielding a saturated alcohol 4, $C_{25}H_{50}O$, with molecular ion at *m/e* 366. Its PMR spectrum ($DCCl_3$) showed a sharp singlet at 8·88 τ  superimposed on a complex of multiplets in the region 8·0 to 9·25 τ, and no signals below 8·0 τ.



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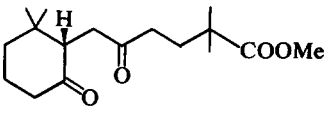
Isodiumycinol, therefore, had to contain a ring and four olefinic bonds. On the basis of this information and the similarity of the spectral data to those found for the isomeric alcohol, isomoenocinol (5),[4] it was possible to assign structure 2 tentatively to isodiumycinol.

Confirmatory evidence that 2 is the correct structure was provided by two degradation schemes. In one, degradation of isodiumycinol (1, $KMnO_4$—$NaIO_4$; 2, CrO_3—H^+; 3, CH_2N_2) gave a mixture of crude methyl esters. Preparative glc yielded the major component as a colorless oil having $[\alpha]_D^{25} + 8°$ ($c = 0{\cdot}30$ g/100 ml EtOH), analyzing by elemental analysis and mass spectrometry (*m/e* 296·1996, H+) for $C_{17}H_{28}O_4$, and having spectral properties consistent with the diketoester structure 6: IR (neat) 1728 (ester C=O), and 1706 cm^{-1} (ketone C=O), but no OH or olefin bands; PMR ($DCCl_3$, 60 MHz) τ 9·27 (s, 3, CH_3—), 8·97 (s, 3, CH_3—), 8·82 [s, 6, $(CH_3)_2$-C—COO], 6·33 (s, 3, ester MeO—), 7–9 (m, 13, methylenes), no signals below 6·33 τ.



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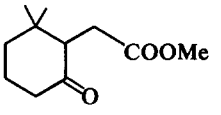

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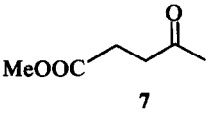

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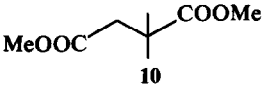

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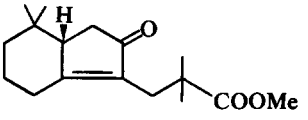

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Prominent peaks in the high-resolution mass spectrum of this diketoester are rationalized as in Fig 1.

In a second degradation of isodiumycinol employing the sequence (1, $KMnO_4$—$NaIO_4$; 2, Ag_2O—NaOH; 3, CH_2N_2), or alternatively the scheme (1, O_3; 2, Zn—AcOH; 3, Ag_2O—NaOH; 4, CH_2N_2), two major products were isolated by

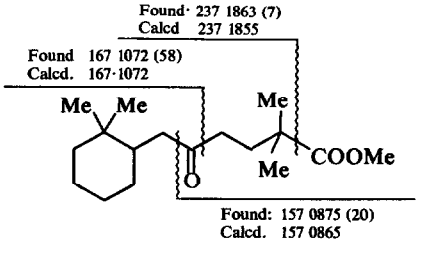
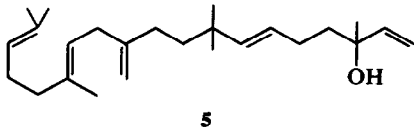


Fig 1.



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preparative GLC. The faster moving major component was identified as methyl levulinate (7) by comparison of its GLC retention times on two different columns and its PMR and IR spectra with those of an authentic sample. The slower moving major component, which was assigned structure **8**, was apparently formed by aldol condensation of a diketone of type **6**. It analyzed for $C_{17}H_{26}O_3$ by high-resolution mass spectrometry and had: $[\alpha]_D^{25}$ +2·8° (EtOH), UV_{max} (EtOH) 239 nm (ε 12,000); IR (neat) 1730 (ester C=O), 1700 and 1640 cm^{-1} (C=C—C=O); PMR ($DCCl_3$) τ 9·33 (s, 3, CH_3—), 9·00 (s, 3, CH_3—), 8·84 [s, 6, $(CH_3)_2$-CCOO], 7·53 (s, 2, unsplit allylic methylene), 6·33 (s, 3, ester MeO—), and no signals below 6·33 τ.

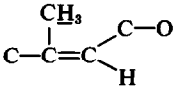
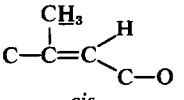
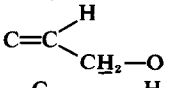
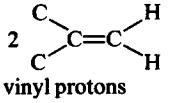
Evidence to support structure **8** was obtained by its conversion in the scheme (1, O_3—CH_2Cl_2; 2, HIO_4; 3, CH_2N_2) to 2,2-dimethylsuccinic acid dimethyl ester (**10**) and the keto ester **9**, which were both isolated by preparative GLC. Dimethylsuccinic acid dimethyl ester was readily identified by comparison of PMR, IR and GLC with those of an authentic sample. The keto ester **9** was identified by comparison of its mass spectrum, GLC retention times, and 2,4-dinitrophenylhydrazone derivative with those of an authentic synthetic sample. An authentic sample of keto ester **9** was obtained from the known ketal alcohol **11**[6,7] by the conversions shown in Scheme I.

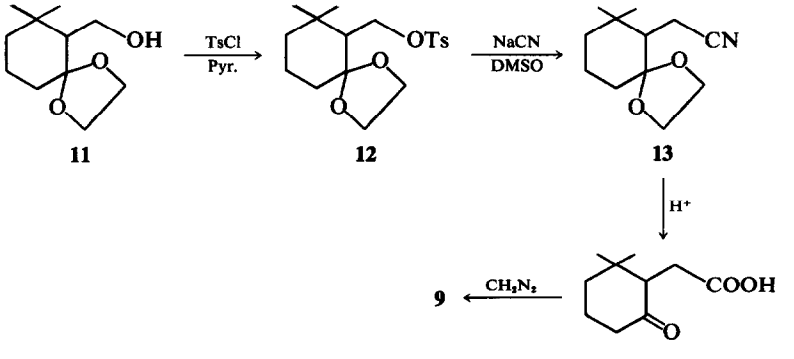
Diumycinol (**1**)

Molecular distillation of diumycinol gave a colorless oil having $[\alpha]_D^{26}$ + 5·6° (*c* = 1·0 g/100 ml EtOH) and analyzing for $C_{25}H_{42}O$ by elemental analysis and mass spectrometry, *m/e* 358 (M+). Its IR spectrum (neat) showed bands at 3050–3600 (OH), 1640 and 1660 (C=C), 1360 and 1380 (Me—C—Me), and 890 cm^{-1} (terminal methylene), whereas its ultraviolet spectrum (EtOH) showed no absorption above 205 nm (no conjugated double bonds). Its PMR spectrum ($DCCl_3$) exhibited signals that are assigned in Table 2.

Hydrogenation of diumycinol with Adams catalyst in ethanol consumed four equivalents of hydrogen to give a saturated alcohol analyzing for $C_{25}H_{50}O$ by mass spectrometry. Treatment of diumycinol with acetic anhydride in pyridine gave, after molecular distillation, an oily acetate that analyzed for $C_{27}H_{44}O_2$ by elemental analysis and mass spectrometry, *m/e* 400 (M+). The IR spectrum ($CHCl_3$) of diumycinol acetate showed the expected bands at 1730 (C=O) and 1240 cm^{-1} (C—O—C), whereas its PMR spectrum ($DCCl_3$) showed a strong singlet at 8·00 τ (CH_3—C(=O)—O—) superimposed on multiplets, and a broad doublet superimposed on a multiplet in the region 5·20–5·6 τ (5–6H, C≡C—CH_2—O—C(=O)—C and 2 C=C—H). The data for diumycinol, its perhydro derivative, and its acetate derivative, in conjunction with the

Table 2

Signal	Assignment
τ 9·13 (s, 3H)	C—CMe_2C
τ 9·05 (s, 3H)	
τ 9·02 (s, 6H)	C—CMe_2—C=C
τ 8·32 (broad singlet, 1–2H)	 *trans*
τ 8·25 (broad singlet, 1–2H)	 *cis*
τ 7·7–8·8 (m, ~ 20H)	methylenes
τ 5·88 (broad doublet, 2H, *J* = 7, *J* = 2)	
τ 5·20–5·55 (m, 3–4H)	
τ 4·6 (m, 3–4H)	vinyl protons



Scheme 1

structural assignment of isodiumycinol, indicated that diumycinol should be represented by structure 1. As expected, mixtures of diumycinol and isodiumycinol on degradation by the scheme (1, O_3; 2, Zn—AcOH; 3, Ag_2O—NaOH; 4, CH_2N_2; 5, O_3; 6, HIO_4—AcOH; 7, CH_2N_2) yielded only 2,2-dimethylsuccinic acid and the ketoester 9 as major products.

Diumycene (3)

Diumycene, when purified by chromatography over neutral alumina using hexane-benzene, analyzed for $C_{25}H_{40}$ by mass spectrometry, *m/e* 340 (56), molecular ion. The IR spectrum ($CHCl_3$) of diumycene showed bands at 1600 and 1640 (sharp) (C=C), 1360 and 1380 [—C—CMe_2C], and 895 (very intense, terminal methylene), while its UV spectrum (EtOH) exhibited λ_{max} 225 nm which suggested a conjugated olefin or a mixture of conjugated olefins, as in structure 3. Its PMR spectrum ($DCCl_3$) showed complicated sets of signals in the region 4·5–5·6 τ (vinylic protons) and in the region 7·65–9·2 τ (methylene and Me protons). Reduction of diumycene using Adams catalyst in hexane-acetic acid consumed five equivalents of hydrogen after 2 hr to give, after molecular distillation, a colorless oil that analyzed for $C_{25}H_{50}$ by elemental analysis and mass spectrometry, *m/e* 350, molecular ion. The data for diumycene and its perhydro derivative, in conjunction with those for diumycinol and isodiumycinol, indicated that diumycene should be represented as a mixture of hydrocarbons 3 resulting from dehydration of diumycinol or isodiumycinol during acid hydrolysis of diumycin. As anticipated, degradation of diumycene by the scheme (1, O_3; 2, Zn—AcOH; 3, CrO_3—H^+; 4, CH_2N_2) gave the diketoester 6, whereas degradation by the method (1, O_3; 2, Zn—AcOH; 3, Ag_2O—NaOH; 4, CH_2N_2; 5, O_3; 6, HIO_4; 7, CH_2N_2) afforded the ketoester 9, isolated as its 2,4-dinitrophenylhydrazone derivative.

Configuration at C-6 *and* C-7 *in the diumycin lipid*

The stereochemistry at C-6 and C-7 in diumycinol and isodiumycinol should be *trans*, since the IR spectra (neat) of these alcohols show no band in the region 840–670 cm^{-1} where a *cis* dialkyl-substituted olefin should absorb. Absorption found at 975 cm^{-1} in both diumycinol and isodiumycinol is consistent with a *trans* configuration at C-6 and C-7.

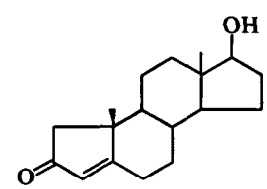
Configuration at C-2 *and* C-3 *in the diumycin lipid*

The chemical shift of the C-3 Me protons at 8·27 τ superimposed on broad multiplets in the PMR spectrum of diumycinol indicates that diumycinol is predominantly the 2-*cis* isomer, whereas a weaker signal at 8·32 τ indicates the presence of the 2-*trans* isomer.[8] It is likely that the diumycin lipid is conjugated to the remainder of the antibiotic in an allylic oxygen linkage, but the configuration around the C-2 double bond in the parent antibiotic is still uncertain, since isomerization may have occurred during hydrolysis.

Absolute configuration at C-13 *in the diumycin lipid*

The ORD curve of the diketoester 6 showed a complex pattern in the region 300–315 nm exhibiting two positive Cotton effects with: maxima $[\phi]^{27}_{312}$ + 1470°; $[\phi]_{302}$ + 1380°; troughs $[\phi]_{306}$ + 1250°; $[\phi]_{268}$ − 1220°; λ_0 288 nm. A simple application of the octant rule for cyclohexanones would predict a single positive Cotton effect for a cyclohexanone of type 6 that was not complicated by an acylic keto function two carbons removed from the asymmetric center. Without a suitable diketone as a model, however, an unambiguous interpretation of the ORD curve of the diketone 6 was not possible.

The indenone 8 provided an alternative possibility for determination of the absolute configuration at C-13 in the diumycin lipid. The low rotation of the indenone suggested that considerable racemization had occurred during its formation by base-catalyzed condensation of a diketone of type 6. Examination of the ORD curve in indenone 8, however, showed that it had a pattern similar, but opposite in direction, to that of (10R)-A-nortestosterone (15)[9] and (8s)-$\Delta^{3,9}$-8-methylhydrinden-2-one (16).[10] The ORD curve of 8 (*c* 0·09, dioxane) had: $[\phi]^{27}_{400}$ + 14°, $[\phi]_{350}$ + 117°, $[\phi]_{345}$ + 122°, $[\phi]_{335}$ + 89°, $[\phi]_{33}$ + 45°, $[\phi]_{326}$ 0°, $[\phi]_{320}$ − 45°, $[\phi]_{305}$ − 167°. The indenone 8 and, therefore, the diketone 6 should both have the (S) configuration, and the diumycin lipids, 1, 2, and 3, should all have the (13S) configurations shown.



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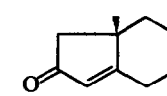

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The nonisoprenoid pattern from C-5 to C-11 in both the diumycin and moenomycin lipids represents a unique structural feature that has not been observed for lipids obtained from either plant or microbial sources. This anomaly suggests the possibility of an unusual biogenesis in which not all of the C atoms are derived from mevalonate.

EXPERIMENTAL

The PMR spectra were determined on Varian NMR spectrometers (Models A-60 and T-60), and IR spectra were recorded on Perkin-Elmer spectrophotometers (Model 257 and Infracord). The mass spectra were obtained on an AEI MS-9 mass spectrometer, and the UV spectra on a Perkin-Elmer Model 202 spectrophoto-

meter. The GLC's were performed on a Hewlett-Packard Model 5750 gas chromatograph equipped with a flame-ionization detector and an effluent splitter. M.ps are uncorrected. ORD measurements were performed by Dr. J. P. Casey, Princeton University.

Acid hydrolysis of diumycin mixture

A soln of diumycin (2·96 g ammonium salt) in 30 ml 1N HCl was heated in an oil bath at 105°, for 30 min. The mixture was cooled to room temp and extracted with chloroform. The chloroform extract was dried (Na_2SO_4) and evaporated *in vacuo* to a dark oil that was applied to five 20 × 20-cm silica-gel HF plates (1 mm thick). The plates were developed in benzene : $CHCl_3$: MeOH (8 : 1 : 1), and the bands were eluted with $CHCl_3$ to give diumycinol (54 mg, R_f 0·68), isodiumycinol (95 mg, R_f 0·80), and diumycene (157 mg, R_f 0·95).

Diumycinol (1)

Diumycinol, after molecular distillation at 95–105° and pressure less than 10^{-3} mm, gave a colorless oil having: $[\alpha]_D^{26} + 5·6°$ ($c = 1·0$ g/100 ml EtOH); mass spectrum, complex but with *m/e* 358(5) molecular ion, 343(2), 340(9), 271(13), 194(39), 123(49), and 69(100) base. (Found: C, 83·57; H, 11·77. Calcd. for $C_{25}H_{42}O$: C, 83·73; H, 11·81%.) The spectral properties of diumycinol have already been described.

Perhydrodiumycinol

Diumycinol (21 mg) was added to Adams catalyst (13 mg) that had been prereduced in 5 ml of 95% EtOH. Hydrogenation for 24 hr at 25° and 1 atm consumed four equivs of H_2. The catalyst was removed by filtration, and the filtrate was evaporated to a residue (20 mg) that was purified by molecular distillation at 95–100° and pressure less than 10^{-3} mm, to give a colorless oil whose IR spectrum ($CHCl_3$) showed no absorptions in the region 1700–1550 cm^{-1} (no C=C) and no bands between 1000–800 cm^{-1} (no terminal methylenes). Its mass spectrum showed prominent peaks at *m/e* 366(4) molecular ion, 237(4), 236(14), 195(5), 194(14), 125(57), 125(75), and 69(100) base.

Diumycinol acetate

To a soln of distilled diumycinol (15 mg) in 3 ml of dry pyridine at 0° was added 0·3 ml of Ac_2O. The mixture was kept at 5° for 3 days. Ice was added to the mixture, and after 5 min, the mixture was diluted with water and extracted 3 times with benzene. The benzene extract was washed with water, dried (Na_2SO_4), and evaporated to a residue. Benzene was added and removed under reduced pressure, and the procedure was repeated until no odor of pyridine could be detected. The pyridine-free residue (18 mg) was distilled at 95–100° and pressure less than 10^{-3} mm, to give 16 mg of diumycinol acetate as a pale-yellow oil with spectral properties already described. (Found: C, 81·13; H, 11·12. Calcd. for $C_{27}H_{44}O_2$: C, 80·94; H, 11·07%.)

Isodiumycinol (2)

A sample of isodiumycinol (25 mg) from TLC was distilled at 80–85° and pressure less than 10^{-3} mm, to give a colorless oil having spectral properties already described, and analyzing for $C_{25}H_{42}O$, by mass spectrometry [*m/e* 358(2), molecular ion] and elemental analysis. (Found: C, 84·04; H, 12·55. Calcd. for $C_{25}H_{42}O$: C, 83·73; H, 11·81). Its mass spectrum was nearly identical to that of diumycinol, with differences found only in the intensities of the peaks.

Perhydroisodiumycinol

A. *Hydrogenation in ethanol.* Isodiumycinol (20 mg) in 5 ml of 95% EtOH was added to Adams catalyst (10 mg) that had been prereduced in 4 ml of 95% EtOH. Hydrogenation for 20 hr at 24° and 1 atm consumed four equivs of H_2 to give, after filtration and evaporation, a colorless oil with spectral properties already described. Molecular distillation of recovered spectral materials gave a colorless oil. (Found: C, 81·44; H, 14·22. Calcd. for $C_{25}H_{50}O$: C, 81·89; H, 13·75%.)

B. *Hydrogenation in acetic acid.* To Adams catalyst (11 mg) prereduced in 5 ml AcOH, was added distilled isodiumycinol (24 mg) in 5 ml AcOH. Hydrogenation for 2 hr at 27° and 1 atm consumed four equivs of H_2. Removal of the catalyst by filtration, and evaporation of the AcOH under reduced pressure, gave a residue that was distilled at 90° and pressure less than 10^{-3} mm, yielding a colorless oil (21 mg) having a mass spectrum like that found for perhydroisodiumycinol obtained by reduction in EtOH. The oil had an IR spectrum ($CHCl_3$) showing no absorption in the region 1700–1550 cm^{-1} (no C=C). (Found: C, 81·93; H, 13·68. Calcd. for $C_{25}H_{50}O$: C, 81·89; H, 13·75.)

Diumycene (3)

Diumycene (16 mg) obtained from silica-gel chromatography had IR and PMR spectral properties already described. Rechromatography of the combined spectral samples on a neutral alumina column (Woelm, 1·5 × 5 cm), using hexane, gave an oil having an IR spectrum identical to that obtained by silica-gel chromatography. The oil gave a mass spectrum with molecular ion *m/e* 340(56) and base peak *m/e* 94. (Found: C, 87·57; H, 11·76. Calcd. for $C_{24}H_{40}$: C, 88·16; H, 11·84%.)

Perhydrodiumycene

Diumycene (12 mg) in 4 ml of AcOH-hexane (1 : 1) was added to Adams catalyst (12 mg) that had been prereduced in 2 ml of AcOH-hexane (1 : 1). Hydrogenation for 2 hr at 25° and 1 atm consumed five equivs of H_2. The catalyst was removed by filtration, and the solvents were removed under reduced pressure to give a residue that was distilled at 80° and pressure less than 10^{-3} mm, yielding a colorless oil with: IR spectrum ($CHCl_3$) showing no absorptions in the region 1700–1550 cm^{-1} (no C=C) or the region 1000–8000 cm^{-1} (no terminal methylenes); mass spectrum showing prominent peaks at *m/e* 350(7) molecular ion, 237(14), 236(34), 125(81), and 69(100) base peak. (Found: C, 85·17; H, 14·65. Calcd. for $C_{25}H_{50}$: C, 86·63; H, 14·37.)

Degradation of isodiumycinol (2) *to indenone* (8) *and methyl levulinate* (7) *via permanganate-periodate oxidation*

To a stirred soln of isodiumycinol (95 mg, 0·27 mmol) in 30 ml of *t*-BuOH was added a soln of $NaIO_4$ (2·20 g, 10·3 mmol) in 25 ml water, followed by a soln of K_2CO_3 (550 mg) and $KMnO_4$ (12 mg, 0·076 mmol) in 5 ml water. The mixture was stirred for 15 hr and filtered. The ppt was washed with *t*-BuOH—H_2O (1 : 1), and the combined filtrate and washings were evaporated to a residue. The residue, after acidification with 1N HCl, was diluted with saturated NaCl soln and extracted with ether. Evapora-

tion of the dried ($MgSO_4$) ether extract gave a residue (90 mg).

The residue and freshly prepared Ag_2O in NaOHaq [$AgNO_3$ (750 mg), NaOH (500 mg), and H_2O (5 ml)] was maintained at 50–60° for 1 hr by occasional warming over a steam bath. When the mixture had reached room temp, the silver sponge was removed by filtration and washed with water. The filtrate was acidified with 10% HCl and extracted with ether. Evaporation of the dried ($MgSO_4$) ether extract gave a residue that was esterified with excess ethereal diazomethane, yielding 90 mg methyl esters.

Preparative GLC on $\frac{1}{4}'' \times 6'$ stainless steel 3% SE-30 column (program: 150 $\xrightarrow{4°/min}$ 200°, isothermal @ 200° for 5 min, immediate increase to 250° and isothermal @ 250°) gave **7** (4 mg, retention time 1 min) and indenone **8** (5 mg, retention time 16·5 min) as the major products. A large portion of both compounds seemed to be lost as an aerosol. The sample of methyl levulinate had IR and PMR spectra identical with those of an authentic sample, and GLC retention times identical with those of authentic methyl levulinate on the 3% SE-30 column and on a $\frac{1}{4}'' \times 6'$ stainless steel 15% Carbowax 20M-2% TPA column (program: 150 $\xrightarrow{6°/min}$ 215°). The indenone had: $[\alpha]_D^{25}$ +2·8° (EtOH); UV max (EtOH) 239 nm (ϵ 12,000), IR (neat) 1730 (ester C=O) 1700 and 1640 cm^{-1} (C=C—C=O); PMR ($DCCl_3$) τ 9·33 (s, 3, CH_3—), 9·00 (s, 3, CH_3—), 8·84 [s, 6, $(CH_3)_2C$—COO], 7·53 (s, 2, unsplit allylic methylene), and 6·33 (s, 3, ester CH_3O—); mass spectrum molecular ion 278·1924(74); calcd. for $C_{17}H_{26}O_3$: 278·1967.

Degradation of isodiumycinol (**2**) *to indenone* **8** *and methyl levulinate* (**7**) *via ozonolysis*

O_3 (Welsbach Generator) was passed through a soln of isodiumycinol (110 mg) in CH_2Cl_2, cooled in an ice-water bath, until several seconds after the color of I_2 appeared in an exit trap containing 2% KI. Evaporation of the CH_2Cl_2 gave a glassy yellow residue that was taken up in 10 ml of AcOH and stirred with Zn dust (750 mg) for 1 hr. The mixture was filtered, and the filtrate was evaporated to a residue that was treated with freshly prepared Ag_2O in NaOH aq [$AgNO_3$ (700 mg), NaOH (600 mg), and H_2O (10 ml)], maintained at 50–60° for 1 hr by occasional warming on the steam bath. The silver sponge was removed by filtration and washed with water. The combined filtrate was acidified with 10% HCl, diluted with satd NaCl aq, and extracted with ether. The ether extract was dried ($MgSO_4$) and evaporated to give a mixture of acids. The mixture was taken up in 5 ml of Et_2O, and treated for several min with excess ethereal diazomethane. Removal of the ether under reduced pressure gave a mixture of methyl esters as a yellow residue (100 mg). Preparative GLC on a 3% SE-30 column, as previously described, yielded 4 mg of **7** and 4 mg of indenone **8**.

Degradation of diumycene (**3**) *to dikeoester* **6** *via ozonolysis*

Diumycene (80 mg), in 40 ml of CH_2Cl_2 of 0°, was treated with O_3 for approximately 4 min until the brown color of iodine appeared in an exit trap of aqueous 2% KI soln. Based on iodometric determination of the amount of O_3 produced per min, the diumycene sample consumed 5–6 equivas of O_3. The CH_2Cl_2 was removed under reduced pressure, and the residue was taken up in 15 ml AcOH, stirred with Zn dust (750 mg) for 1 hr, and filtered. Evaporation of the filtrate gave a residue that was taken up in $CHCl_3$–H_2O. The $CHCl_3$ layer was dried ($MgSO_4$) and evaporated to another residue.

A stirred acetone soln (15 ml) of the residue was cooled in an ice-water bath and treated with approximately 0·8 ml of CrO_3 soln [CrO_3 (404 mg), and H_2SO_4 (2 ml), diluted with water to 10 ml] until an orange color was discernible. MeOH (3 ml) was added, followed by H_2O (5 ml). MeOH and acetone were removed under reduced pressure, and water (20 ml) was added. The soln was extracted with ether (four 40-ml portions), and the ether was washed with a small amount of water, dried ($MgSO_4$), and evaporated to a residue. Esterification of the residue with Et_2O—CH_2N_2 yielded 50 mg of methyl esters.

Preparative GLC on a $\frac{1}{4}'' \times 6'$ stainless steel 3% SE-30 column (program: 165 $\xrightarrow{6°/min}$ 220°, then isothermal at 220°) gave **6** (6 mg, retention time 11·5 min). A large amount of the diketoester seemed to be lost as an aerosol.

The diketoester had: $[\alpha]_D^{25}$ + 13° (EtOH); IR (neat) 1728 (ester C=O), 1706 cm^{-1} (C=O); PMR ($DCCl_3$)τ9·27 (s, 3, CH_3—), 8·97 (s, 3, CH_3—), 8·82 [s, 6, $(CH_3)_2C$—COO], 6·33 (s, 3, ester MeO). (Found: C, 69·04; H, 9·68; molecular ion 296·1996. Calcd. for $C_{17}H_{28}O_4$: C, 68·89; H, 9·52; molecular ion 296·1988.)

Degradation of isodiumycinol (**2**) *to diketoester* **6** *via permanganate periodate oxidation*

To a soln of isodiumycinol (85 mg, 0·24 mmol) in 30 ml of t-BuOH was added a soln of $NaIO_4$ (2·20 g, 10·3 mmol) in 25 ml H_2O, followed by a solution of $KMnO_4$ (12 mg, 0·076 mmol) and K_2CO_3 (550 mg) in 5 ml H_2O. The mixture was stirred for 15 hr and evaporated to a residue that was taken up in satd NaCl aq, acidified with 10% HCl, and extracted repeatedly with ether. The ether extract was dried ($MgSO_4$) and evaporated to a residue.

A stirred soln of the residue in 15 ml acetone was cooled in an ice-water bath and treated dropwise with chromic acid soln (~ 1 ml), described previously, until an orange color was discernible. A 50% MeOH—H_2O soln (5 ml) was added, and the mixture was stirred for several min. MeOH and water were removed under reduced pressure, and the mixture was extracted with ether. The ether extract, after being dried ($MgSO_4$) and evaporated, yielded a residue that was esterified with excess ethereal diazomethane to give 45 mg methyl esters. Preparative GLC on a $\frac{1}{4}'' \times 6'$ stainless steel 3% SE-30 column, under conditions previously described, gave **6** (5 mg) as a colorless oil.

Degradation of indenone **8** *to ketoester* **9** *and* 2,2-*dimethylsuccinic acid dimethyl ester* **10**.

A soln of **8** (5 mg from preparative GLC) in 10 ml CH_2Cl_2 was cooled in a Dry Ice-acetone bath and ozonized for several seconds until the yellow-brown color of I_2 appeared in an exit trap of aqueous 2% KI. The pale-violet CH_2Cl_2 soln was flushed immediately with N_2, allowed to warm to room temp, and concentrated to a 0·5-ml volume. Aqueous 0·33M HIO_4 (~ 0·4 ml) was added followed by sufficient AcOH (~ 0·5 ml) to effect a homogeneous soln. The stoppered soln was stirred for 16 hr at room temp, treated with satd NaCl aq, and extracted repeatedly with CH_2Cl_2. The CH_2Cl_2–AcOH extract was dried (Na_2SO_4), and evaporated under reduced pressure. Esterification of the residue with Et_2O—CH_2N_2 and careful removal of the ether under reduced pressure gave 1 mg of methyl esters.

The methyl ester mixture showed two major components on GLC. On a $\frac{1}{4}''\times 6'$ stainless steel 3% SE-30 column (program: 125 $\xrightarrow{10°/min}$ 200°), one component had the same retention time (1·8 min), on admixing, as **10**, whereas the other major component had the same retention time (5·1 min) on admixing as the authentic ketoester **9**. Similarly, on a $\frac{1}{4}''\times 6'$ stainless steel 15% Carbowax 20M–2% terephthalate column (program: 150 $\xrightarrow{10°/min}$ 210° and isothermal at 210°), the faster moving major component had the same retention time (6·1 min), on admixing, as **10**, whereas the slower moving major component had the same retention time (16·5 min) on admixing as the authentic ketoester **9**.

Degradation of diymycene (**3**) *to ketoester* **9**, *and isolation of its* 2,4-*dinitrophenylhydrazone derivative*

O_3 was passed through a soln of diumycene (400 mg) in 40 ml CH_2Cl_2 at 0° until the yellow-brown color of iodine appeared in an exit trap of 2% KI aq. The CH_2Cl_2 was removed under reduced pressure yielding a glassy residue that was dissolved in 15 ml AcOH and stirred with Zn dust (2·0 g) for 1 hr. The mixture was filtered, and the filtrate was evaporated to a residue that was taken up in $CHCl_3$, filtered, and evaporated to a second residue. The residue was treated with freshly prepared Ag_2O in NaOH aq [$AgNO_3$ (2·25 g), NaOH (2·00 g), and H_2O (15 ml)], maintained at 50–60° for 1 hr by occasional warming on the steam bath. The silver sponge was removed by filtration and washed with water. Acidification (10% HCl) of the filtrate and extraction with ether gave, after drying ($MgSO_4$), a residue that was converted with Et_2O–CH_2N_2 to an orange-red oil (261 mg).

A soln of the oil (100 mg) in 20 ml CH_2Cl_2 at 0° was treated with a stream of O_3 for 5 min. The soln was flushed with N_2 and the solvent was removed under reduced pressure, yielding a residue that was taken up in 2 ml CH_2Cl_2. After addition of 0·33M HIO_4 (8 ml) and sufficient AcOH (8–9 ml) to effect soln, the stoppered flask was stirred for 16 hr at room temp; then the soln was diluted with satd NaCl aq, and extracted repeatedly with CH_2Cl_2. Evaporation of the dried (Na_2SO_4) CH_2Cl_2–AcOH soln left a residue that was esterified with Et_2O–CH_2N_2. Careful evaporation of the filtered ethereal soln gave a mixture of oily methyl esters (67 mg).

A soln of the methyl esters in 95% EtOH was treated with several drops (excess) of filtered 2,4-dinitrophenylhydrazine soln [2,4-dinitrophenylhydrazine (500 mg), conc HCl (1 ml), and 95% EtOH (10 ml)]. The resulting ppt was collected by filtration, dried, and applied to two silica-gel plates (20 × 20 cm PQIF-Quantum). Development of the chromatograms in the system benzene: EtOAc: MeOH (22:1:1) and elution ($CHCl_3$) of the band with R_f 0·9 gave a solid (~ 30 mg) containing no residual 2,4-dinitrophenylhydrazine reagent. Preparative TLC of this solid on one PQIF silica-gel plate using benzene: EtOH (11:1), and elution ($CHCl_3$) of a narrow UV-fluorescing band in the broad orange band with R_f ~ 0·9, gave a residue (8 mg) that yielded clusters after slow crystallization from $CHCl_3$. Recrystallization of the clusters from 95% EtOH gave 2·5 mg of feathery crystals, mp 168–170°, that showed no mixture-m.p. depression on admixing with the 2,4-dinitrophenylhydrazone of authentic ketoester **9**. The IR spectrum (KBr) and mass spectrum of these crystals were identical with those of the 2,4-dinitrophenylhydrazone derivative of authentic ketoester **9**.

Degradation of diumycinol (**1**) *and isodiumycinol* (**2**) *to* 2,2-*dimethylsuccinic acid dimethyl ester* (**10**) *and ketoester* **9**

A 400-mg mixture of diumycinol and isodiumycinol was converted to 176 mg of methyl esters by the procedure (1. O_3, 2. Zn–AcOH, 3. Ag_2O–NaOH, 4. CH_2N_2) previously described for degradation of 400 mg of diumycene. Ozonolysis of 170 mg of this methyl ester mixture in 30 ml CH_2Cl_2 at 0° for 5 min, and subsequent treatment of the product in CH_2Cl_2 (3–4 ml) with aqueous 0·33M HIO_4 and AcOH (9 ml), as described previously gave, after esterification with Et_2O–CH_2N_2, 154 mg methyl esters. Preparative GLC on a $\frac{1}{4}''\times 6'$ stainless steel Carbowax 20M–2% terephthalate column (program: 130 $\xrightarrow{6°/min}$ 220° and isothermal at 220°), gave **10** (4 mg, retention time 6·0 min), and **9**, (6 mg retention time 16·5 min). The GLC sample of **10** had IR and PMR spectra identical with those for an authentic synthetic sample. The GLC sample of the ketoester had IR and PMR spectra identical with those for an authentic sample. The GLC sample of the ketoester had $[\alpha]_D = 0°$ (*c*, 0·4, $CHCl_3$).

The degradation scheme above was repeated with 170 mg diumycinol in place of the mixture of diumycinol and isodiumycinol. Initial ozonolysis was terminated 1 min after the appearance of iodine in a 2% KI trap. Reduction was carried out with Zn dust (2 g) in 15 ml AcOH, and Ag_2O treatment was performed with [$AgNO_3$ (1·5 g), NaOH (1·5 g), and H_2O (10 ml)]. The second ozonolysis was carried out for 5 min, and the subsequent oxidation was performed with [CH_2Cl_2 (3 ml), 0·33M HIO_4 (10 ml), and AcOH (10 ml)]. The oily mixture of methyl esters (139 mg) was subjected to GLC on a $\frac{1}{4}''\times 6'$ stainless steel 3% SE-30 column (program: 125 $\xrightarrow{10°/min}$ 200°). The major peak (retention time 5·0 min) yielded a colorless oil having a mass spectrum identical with that for a sample of authentic **9**.

Tosylate of 2-*hydroxymethyl*-3,3-*dimethylcyclohexanone*-1 *ethylene ketal* (**12**)

The ketal **11** was obtained in 30% overall yield by known procedures[6,7] starting from mesityl oxide. All of the intermediates, with the exception of the dimedone carboxylic acid ethyl ester, were purified by distillation. All b.ps were in agreement with those reported, and PMR and IR spectra of all compounds were consistent with expected signals.

To a soln of **11** (600 mg, 3 mmol) in 1·5 ml dry pyridine at 0° was added p-toluenesulfonyl chloride (684 mg, 3·2 mmol). The mixture was kept at 5° for 17 hr. EtOAc (1 ml) was added, followed by cold 5% $NaHCO_3$ aq (1 ml). After 2 min, 5% $NaHCO_3$ (30 ml) was added, followed by cold EtOAc (50 ml). The aqueous layer was separated and extracted several times with $CHCl_3$. The $CHCl_3$ extract was combined with the EtOAc layer, dried (Na_2SO_4), and evaporated under reduced pressure to a small volume. Benzene (30 ml) was added, and the soln was evaporated to a small volume again. Further quantities of benzene were added and removed until no odor of pyridine could be detected. The residue was dried under vacuum, yielding a colorless oil (1·00 g, 95% yield) that crystallized easily on standing at room temp. The crystalline product had PMR and IR spectra consistent with structure **12**. A sample that was recrystallized from 95% EtOH had mp 69°. (Found: C, 61·27; H, 7·29. Calcd. for $C_{18}H_{26}O_5S$: C, 61·00; H, 7·40.)

2,2-Dimethyl-6-oxocyclohexylacetonitrile ethylene ketal (**13**)

A mixture of **12** (3·51 g, 10 mmol) and NaCN (1·96 g, 40 mmol) in 18 ml dry DMSO was stirred at 70° for 24 hr under N_2. The mixture was cooled to room temp, poured into ice water, and extracted repeatedly with benzene. The benzene extract was washed 4 times with water, and dried (Na_2SO_4). Removal of the benzene under reduced pressure gave **13** (2·02 g, 97% yield) as a pale-yellow oil. Its PMR spectrum ($DCCl_3$) was consistent with the expected signals, showing Me singlets at 9·07τ (3H) and 8·95τ (3H), whereas its IR spectrum ($CHCl_3$) showed a band at 2240 cm^{-1} (—C≡N).

2,2-Dimethyl-6-oxocyclohexylacetic acid (**14**) *and its methyl ester* (**9**)

A mixture of oily **13** (418 mg, 2 mmol) and 1·5 ml of a soln of H_2SO_4:AcOH:H_2O (1:1:1) was refluxed for 2 hr, cooled to room temp, diluted with water, and made basic with 5% $NaHCO_3$. The soln was extracted repeatedly with ether, and the ether layer was discarded. The aqueous layer was acidified with dil HCl, and extracted repeatedly with ether. This ether extract was dried (Na_2SO_4) and evaporated, yielding **14** as an oil (177 mg, 48% yield), that crystallized on standing at room temp. The PMR spectrum ($DCCl_3$) of the acid was consistent with structure **14**, showing a signal at −0·1τ (1H, broad, –COOH), and geminal Me signals at 8·92 and 9·25τ. Its IR spectrum ($CHCl_3$) showed an intense band at 1715 cm^{-1} (—C=O and —COOH). Recrystallization of the product from cyclohexane-benzene gave colorless crystals having m.p. 131–132°, lit, m.p. 131–132°.[11]

Treatment of **14** with ethereal diazomethane gave **9** as an oil that showed PMR singlets at 6·32 (3H, ester —Me), 8·92 (3H, —Me), and 9·25τ (3H, —Me), IR bands ($CHCl_3$) at 1705 and 1725 cm^{-1} (—C=O and —COOMe), and a molecular ion at *m/e* 198 (20).

2,4-Dinitrophenylhydrazone derivative of authentic synthetic 2,2-dimethyl-6-oxocyclohexylacetic acid methyl ester (**9**).

An ethanolic soln of **9** obtained from **13** was treated with an ethanolic-HCl soln of 2,4-dinitrophenylhydrazine. The resulting ppt was collected, dried, and applied to silica-gel plates (PQ1F-Quantum, 20×20 cm, 1 mm thick), that were chromatographed in the system benzene:EtOAc:MeOH (22:1:1). Elution ($CHCl_3$) of the band having R_f 0·9 gave the 2,4-dinitrophenylhydrazone of **9** as as a residue. Recrystallization of this residue from 95% EtOH gave orange needles, m.p. 169–171°, with mass spectrum: *m/e* 378(46), M+; 363(26), M−15; 347(26), M−31; 331(26), M—HONO; 69(100) base. (Found: C, 53·99; H, 5·69; N, 14·85. Calcd. for $C_{17}H_{22}N_4O_6$: C, 53·96; H, 5·86; N, 14·81.)

Acknowledgement—We thank Mr. J. Alicino and his staff for elemental analyses, Dr. A. I. Cohen and his staff for mass spectra and 60-MHz PMR spectra, Mrs. B. Keeler-Toeplitz and her associates for IR spectra, and Dr. J. P. Casey, Princeton University, for ORD measurements.

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