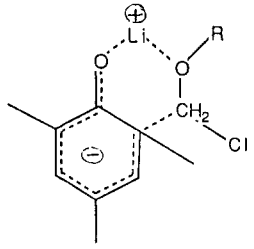
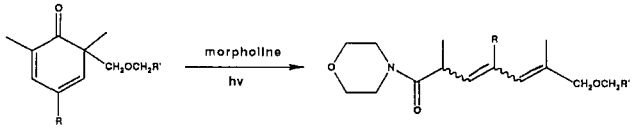


Figure 1.



6	R = CH_3	R' = H	9	R = CH_3	R' = H
7	R = CH_3	R' = C_6H_5	10	R = CH_3	R' = C_6H_5
8	R = CH_2OH	R' = C_6H_5	11 &12	R = CH_2OH	R' = C_6H_5

Figure 2.

2,4,6-Trimethylcyclohexa-2,4-dien-1-one (6). Cyclohexadienone **6** was obtained as per the general procedure from 2,4,6-trimethylphenol and chloromethyl methyl ether. The concentrate was applied on C18 reverse-phase column. By use of a gradient of methanol in water, cyclohexadienone was obtained as a colorless liquid: 65% yield; R_f 0.88 (C18 reverse-phase; MeOH–H_2O, 90:10); ^1H NMR ($CDCl_3$, 200 MHz) δ 1.12 (s, 3 H, CH_3), 1.92 (s, 3 H, CH_3), 1.99 (s, 3 H, CH_3), 3.29 (s, 3 H, OCH_3), 3.33–3.68 (d, 2 H, system AB, J_{AB} = 9 Hz, CH_2), 6.03 (s, 1 H), 6.82 (s, 1 H); UV λ (CCl_4) 315 nm (3436); IR (neat) 2850, 1660 cm^{-1}; MS m/z 180 (M^+, 87), 148 (100), 135 ($M^+ - CH_2OCH_3$, 25). Anal. Calcd for $C_{11}H_{16}O_2$: C, 73.30; H, 8.95. Found: C, 73.54; H, 9.07.

General Procedure for the Photochemical Cleavage of the Cyclohexa-2,4-dienones. The cyclohexa-2,4-dien-1-one (0.2 mmol) dissolved in dry ether (20 mL) was placed in a Pyrex tube. Morpholine (500 μL) was added. Oxygen from the reaction mixture was expelled by bubbling an inert gas. Then the reaction tube was irradiated with UV lamp (Hanovia, medium-pressure, 100 W). The reaction was followed by either UV absorption measurements or TLC. After complete consumption of the starting material, which required nearly 4 h, solvent and the morpholine were removed under reduced pressure, and the concentrate was subjected to flash chromatography.

Photochemical Cleavage of 2,4,6-Trimethylcyclohexa-2,4-dien-1-one (6). Cyclohexadienone **6** was subjected to photochemical cleavage as per the general procedure. After 4-h irradiation, complete consumption of the starting material and appearance of single reaction product were observed. The usual workup afforded diene **9** as a colorless liquid in quantitative yield: R_f 0.22 (ethyl acetate–hexane, 50:50); ^1H NMR ($CDCl_3$, 200 MHz) δ 1.17 (d, 3 H, J = 7 Hz, 10-CH_3), 1.67 (s, 3 H, 8-CH_3), 1.82 (s, 3 H, 9-CH_3), 3.2–3.8 (m, 8 H, morpholine), 3.37 (s, 3 H, OCH_3), 3.40 (dq, 1 H, J = 10 and 7 Hz, 2-H), 3.88 (s, 2 H, CH_2O), 5.40 (d, 1 H, J = 10 Hz, 3-H), 5.93 (s, H, 5-H); UV λ (MeOH) 206 nm (20482); IR (neat) 2850, 1655, 1440, 1200–1000 cm^{-1}; MS m/z 268 ($(M + 1)^+$, 39), 253 (9), 237 ($(M + 1)^+ - OCH_3$, 9), 121 (100), 114 (48).

Acknowledgment. I am grateful to Professor Sir D. H. R. Barton, Professor R. Breslow, and Dr. M. Barbier and his research group (ICSN, CNRS, Gif-Sur-Yvette, France) for their help and useful discussions.

Registry No. 1, 576-26-1; 2, 4397-14-2; 3, 527-60-6; 6, 123674-61-3; 7, 123674-62-4; 8, 123674-63-5; 9, 123674-64-6; 10, 123674-65-7; 11 & 12, 123674-66-8; 13, 123674-67-9; 14, 123674-68-0; 15, 123674-69-1; 16, 123674-70-4; 17, 16184-95-5; 18, 123674-71-5; 19, 123674-72-6; 20, 123674-73-7; 21, 16184-96-6; 22, 123674-74-8; phomenoic acid, 83652-15-7; phomenolactone, 83652-16-8; morpholine, 110-91-8.

Stereochemistry of Hydrogen Loss on Formation of the Vinyl Group in the Biosynthesis of Ravidomycin

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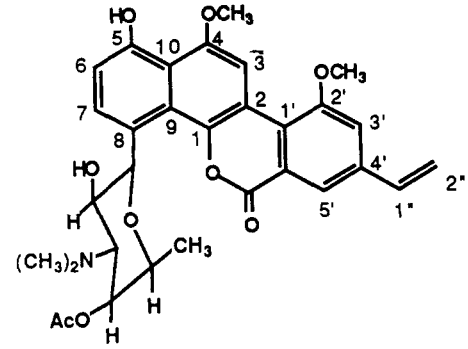
The antibiotic ravidomycin was isolated from *Streptomyces ravidus*[1] and shown to have structure **1** by Findlay and his collaborators.[2] Its aglycone unit is identical with that of the related antibiotics chrysomycin V[3] (albacarcin V,[4] virenomycin V[5]) and toromycin[6] (gilvocarin V[7]).



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Ravidomycin shows significant antitumor activity against P388 lymphocytic leukemia and colon 38 and CD8F1 mammary tumors in mice.[1] The vinyl group appears to be essential for effective antitumor activity. Thus gilvocarcin V, like ravidomycin, shows significant antitumor activity in P388 lymphocytic leukemia,[8,9] but gilvocarcin M, in which the vinyl group is replaced by a methyl group, is significantly less active in this system. Similarly, dihydroravidomycin is less effective against P388 than is ravidomycin, although the difference in this case seems to be associated with increased toxicity of the dihydro antibiotic at higher doses in this assay.[10] Interestingly, it has been shown that gilvocarcin V is activated by low doses of visible light to induce bacteriophage λ in *Escherichia coli*, while gilvocarcin M fails to show this activation even though it has a similar absorption spectrum.[11] These results thus suggest that the vinyl group of antibiotics of the ravidomycin/toromycin/gilvocarcin type is essential for effective antitumor activity.

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Table I. Results of Incorporation Experiments. GC MS Analyses of Dimethyl Phthalate from Ravidomycin Degradation

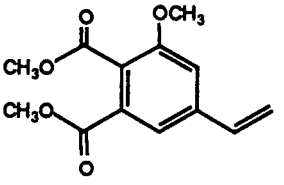
		int of ions in range m/z 219–221[a]						
		uncorr[b]			corr[b,c]			
expt	precursor	219	220	221	219	220	221	% labeled antibiot
1	CH_3CH_2COONa	100	13.2	1.8	100	0	0	0
2	CH_3CD_2COONa	100	22.4	3.1	100	9.2	0	8.4
3	(*R*)-$CH_3CHDCOONa$	100	13.2	1.9	100	0	0.1	0
4	(*R*)-$CH_3CHDCOONa$	100	13.3	1.8	100	0.1	0	0.1
5	(*S*)-$CH_3CHDCOONa$	100	14.3	1.9	100	1.1	0	1.1
6	(*S*)-$CH_3CHDCOONa$	100	14.2	1.9	100	1.0	0	1.0

[a] Error of measurement is ±0.1. [b] Intensities are the average of at least five scans. [c] Ion intensities corrected for signal resulting from ions containing natural-abundance ^{13}C and ^{18}O.

Because of the importance of the vinyl group to the antitumor activity of ravidomycin, a study of its biosynthesis is of significant interest. The basic biosynthetic pathway of ravidomycin has been elucidated,[12] and it is thus known that the vinyl group has its origin in a propionate chain-starter unit. The stereochemistry of formation of the double bond is however unknown. Hence it is of interest to determine whether there is any stereospecificity associated with hydrogen loss from the methylene group of the propionate precursor, and if so, whether the pro-*R* or the pro-*S* proton is preferentially lost on formation of the vinyl group.

Results and Discussion

Determination of the stereospecificity of formation of the vinyl group requires the use of a propionate precursor stereospecifically labeled at the 2-position. Because the published synthesis of labeled propionate[13] was not readily adaptable to a small scale for the preparation of [2-^{3}H]-propionate, we elected to use a ^{2}H label, with isotopic analysis by mass spectrometry.[14] Analysis of intact ravidomycin for deuterium incorporation was not possible due to the errors associated with obtaining isotopic abundances on high-mass ions, but degradation of the antibiotic with alkaline hydrogen peroxide followed by methylation with diazomethane yielded inter alia the dimethyl phthalate **2**,[6] and this could be conveniently ana-



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lyzed by GC MS. Its mass spectrum showed a molecular ion at m/z 250 and a base peak at m/z 219 due to loss of methoxyl from the molecular ion; this base peak was suitable for determination of isotopic abundances.

Stereospecifically deuterated sodium propionate precursors were prepared from (*S*)-(−)-ethyl lactate and (*R*)-(+)-methyl lactate by a modification of the published procedure[13] that avoided the use of a spinning band column to separate triethyl borate and ethyl propionate (or the corresponding methyl esters).

Incorporation of the stereospecifically labeled precursors and of sodium [2,2-$^2H_2$]propionate into ravidomycin was carried out as described in the Experimental Section. The labeled antibiotic was isolated and degraded to a four-component mixture containing the phthalate ester **2**, and GC MS analysis of the phthalate peak allowed determination of the intensity of the ions at m/z 219–221 (Table I).

Incorporation of CH_3CD_2COONa (experiment 2) proceeded very efficiently and gave an antibiotic that was enriched to the extent of 8.4% with deuterium. This experiment thus confirmed the previously published result on the incorporation of propionate into ravidomycin[12] and verified that the analytical methodology employed was appropriate to determine incorporation of deuterium-labeled propionate.

Incorporation of the stereospecifically labeled precursors regrettably proceeded much less efficiently, for reasons that are not entirely clear but are presumably associated with unknown variations in the culture conditions or in the organism. This was true even though each experiment was carried out under conditions that were as similar to those of experiment 2 as possible. Because of the low incorporation observed, each stereospecific incorporation study was carried out twice, to ensure that the low incorporations observed were real and were not due to some statistical error.

The results of these incorporation studies are consistent (Table I). The antibiotic isolated from the (*S*)-propionate precursor (experiments 5 and 6) has retained a small but measurable amount of label, while that from the (*R*)-propionate precursor (experiments 3 and 4) contains no detectable label. We thus conclude that the pro-*S* proton of propionic acid is retained in the biosynthesis of ravidomycin.

The observation of stereospecificity in biosynthesis of ravidomycin leaves open the exact mechanism of formation of the vinyl group. One attractive hypothesis is that the methylene group in an appropriate precursor is hydroxylated, and the hydroxylated precursor then loses water to yield ravidomycin or a precursor thereof. This mechanism would be consistent with the observation of hydroxylated precursors to dehydroamino acids in antibiotics such as berninamycin[15] and virginiamycin M_1.[16] Alternatively, desaturation could occur by a mechanism similar to that observed for the aerobic conversion of saturated fatty acids to monounsaturated fatty acids, in which free hydroxy intermediates do not play any part; this conversion has been shown to be stereospecific and syn.[17]

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Experimental Section

General Methods. General experimental procedures were as previously described.[18] Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Sodium borodeuteride was obtained from MSD Isotopes and was stated to have an isotopic purity of 98 at. % ^2H. ^1H NMR spectra were obtained in D_2O by using dioxane as internal standard.

Sodium (2*R*)-[2-$^2H_1$]Propanoate. Ethyl (2*S*)-2-(*p*-(tolylsulfonyl)oxy)propionate was prepared from (*S*)-(−)-ethyl lactate (Aldrich Chemical Co.) as previously described.[13] This ester 40.4 g, 0.158 mmol) was stirred by magnetic stirrer with sodium borodeuteride (3.3 g, 0.079 mmol) at 100 °C for 48 h under 2–3 mmHg of pressure. The volatile products were collected in a −78 °C trap and identified by GC as ethyl propionate and triethyl borate. This mixture was hydrolyzed with sodium hydroxide (0.6 M in 50% aqueous ethanol) at room temperature for 2 h, and the solution then extracted with ether to remove organic material. The aqueous layer was then acidified with dilute HCl and extracted with ether (3 × 200 mL), and the ether washed (water, brine) and dried. Careful evaporation of the ether gave free propionic acid, which was neutralized to pH 8 with 0.6 M NaOH, and the solution evaporated to give sodium (2*R*)-[2-$^2H_1$]propionate: yield 1.3 g (9.0%); $[\alpha]^{28}_D$ = −0.87° (*c* 6.9, H_2O) [lit.[13] $[\alpha]^{28}_D$ −0.88° (*c* 10, H_2O); ^1H NMR δ 1.01 (dt, 3 H, *J* = 7.7, 0.9 Hz), 2.12 (qt, 1 H, *J* = 7.7, 2.3 Hz).

Sodium (2*S*)-[2-$^2H_1$]Propanoate. (*R*)-(+)-Methyl lactate (Aldrich Chemical Co.) was converted to methyl (2*R*)-2-(*p*-(tolylsulfonyl)oxy)propionate, and this ester (43.1 g) was reduced to sodium (2*S*)-[2-$^2H_1$]propanoate by the method described above: yield 1.90 g (11.7%); $[\alpha]^{28}_D$ + 0.81 (*c* 2, H_2O); ^1H NMR identical with that of the 2*R* isomer.

Culture Conditions. *Streptomyces griseo-olivaceus*, strain C23201-NS7 obtained from Lederle Laboratories, was maintained on agar slants at 4 °C until needed. The mycelium from one slant was transferred to an inoculum medium of 50 mL in each of two baffled 240-mL flasks; the inoculum medium consisted of yeast extract (0.5%), beef extract (0.3%), tryptose (0.5%), dextrin (2.4%), dextrose (0.5%), and $CaCl_2$ (0.4%) in distilled water. Incubation was carried out at 28 °C and on a rotary shaker at 200 rpm for 4 days. A second stage inoculum was then prepared by transferring 5% of the first stage inoculum into each of four flasks containing 50 mL of the same medium and incubating for 3 days at 28 °C and 200 rpm. The second stage inoculum (4 × 10 mL) was then transferred into four 1000-mL baffled flasks containing the fermentation medium: dextrose (1.5%), glycerol (1.5%), soybean flour (1.5%), $CaCO_3$ (0.1%), and NaCl (0.3%). Incubation was carried out for 6 days at 28 °C and 200 rpm, with addition of labeled sodium propionate after 28 h.

Isolation of Ravidomycin. Ethyl acetate (300 mL) was added to each fermentation flask, and the contents were stirred overnight and then filtered through Hyflo SuperCel. The layers were separated, and the aqueous layer was reextracted with EtOAc; the organic extracts were then combined, washed (H_2O, brine), dried (Na_2SO_4), and evaporated. The crude product was purified by flash chromatography (acetone:hexanes 60:40) to yield ravidomycin (100–148 mg) as a yellow powder, homogeneous on TLC (silica gel, acetone:hexanes 60:40) with the same R_f (0.23) as an authentic sample.[12]

Analysis of Deuterium Incorporation. Ravidomycin (50 mg) was dissolved in 5% KOH (1.76 mL) and treated with 3% H_2O_2 (0.44 mL). The solution was stirred at 80 °C for 4 h with addition of three additional portions of H_2O_2 at 1-h intervals. The resulting solution was cooled, acidified with dilute HCl, and extracted with EtOAc (3 × 15 mL). The organic extracts were combined, washed, dried (Na_2SO_4), and evaporated to yield a crude yellow solid. This material was dissolved in 95% EtOH and treated with an ethereal solution of diazomethane until the yellow color persisted. Excess diazomethane was destroyed (AcOH), and the solution was evaporated. The crude product (10 mg) was analyzed by GC MS (HP5 column, 25m × 0.32 mm, 75–200 °C, VG7070E-HF instrument) to yield four major peaks with retention times of 6.85, 7.97, 9.12, and 11.78 min and relative areas of 0.35, 0.30, 0.20, and 0.15. The peak at 7.97 min (estimated yield of 6%) had a mass spectrum consistent with compound **2**: *m/z* 250 (M^+, 13), 219 (100), 187 (15), 160 (10), 145 (7), 118 (3), 102 (8), 77 (7). MS data for the labeled compounds are given in Table I.

Acknowledgment. We thank Dr. Don B. Borders of Lederle Laboratories for gifts of ravidomycin and *S. griseo-olivaceus* strain C23201-NS7.

Registry No. 1, 74622-75-6; 2, 123701-27-9; CH_3CH_2COONa, 137-40-6; CH_3CD_2COONa, 21386-58-3; (*R*)-$CH_3CHDCOONa$, 73493-56-8; (*S*)-$CH_3CHDCOONa$, 123701-28-0; (*S*)-(−)-ethyl lactate, 687-47-8; (*R*)-(+)-methyl lactate, 17392-83-5; ethyl (2*S*)-2-((*p*-tolylsulfonyl)oxy)propionate, 57057-80-4; methyl (2*S*)-2-((*p*-tolylsulfonyl)oxy)propionate, 109579-04-6.

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Stereochemical Aspects of the Additions of Anti-Selective, Crotyl Organometallic Reagents to α-Alkoxy Aldehydes

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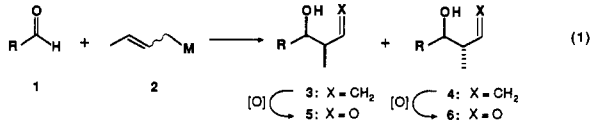
Introduction

The addition of crotyl organometallic reagents **2** to aldehydes **1** according to eq 1 constitutes a useful reaction



(1)

that has been widely exploited in synthetic organic chemistry.[2] This process results in the formation of two contiguous stereogenic centers, and either the syn- or the anti-adducts **3** and **4**, respectively, may be preferentially produced.[2-9] The importance of this reaction lies in the

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