Sparsomycin, Structure and Chemistry[1]

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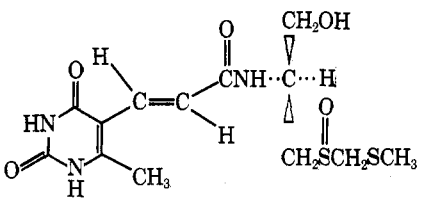
A combination of spectral data and chemical degradation has established the structure of sparsomycin to be that shown in 1.

The isolation of the antibiotic sparsomycin (1) was reported some years ago by Argoudelis and Herr,[2] and a preliminary report discussing its structure has also been published.[3] The present publication presents in detail the evidence for the previously proposed structure (1) and discusses further the chemistry of sparsomycin.



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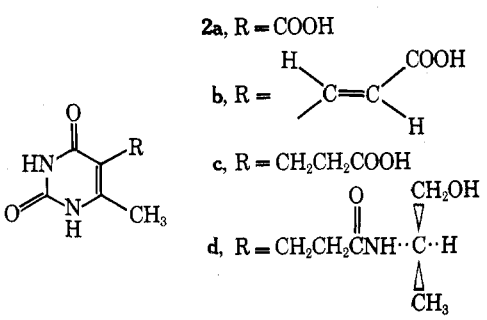
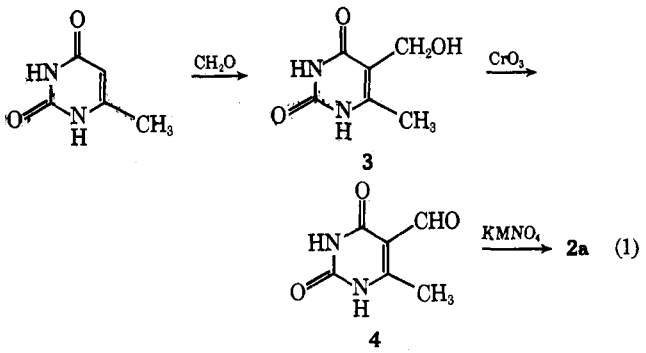
The molecular formula for 1 suggested by Argoudelis and Herr was $C_{13}H_{19-21}N_3O_6S_2$. This suggestion was based on analytical data on material which was subsequently found to be a hydrate. Sparsomycin dried under high vacuum at room temperature for 48 h or more loses 80–90% of its water content. Analyses done on material dried in this way correspond to the molecular formula $C_{13}H_{19}N_3O_5S_2$. The presence of two CH_3C groups was reported, again based on analysis, and acetyl and methoxyl groups were found to be absent. However, subsequent analyses showed the presence of only one CH_3C group although the NMR spectrum of sparsomycin showed the presence of a second methyl group. Titration indicated the presence of a weakly acidic proton.

Oxidation of sparsomycin with aqueous potassium permanganate led to only one product (**2a**) which was an acid having a molecular formula, shown by analysis and mass spectrometry, of $C_6H_6N_2O_4$. The composition and the uv



spectrum, which had a single strong maximum at 267 nm shifting to 288 nm with base, suggested a substituted uracil. Indications of the presence of a carboxyl group in the product by its solubility in weak base and the presence of a methyl group shown by its NMR spectrum first led to the view that the acid was 5-methylorotic acid. Such a consideration was also logical on biogenetic grounds. However, a direct comparison of **2a** with 5-methylorotic acid established that they differed. In such case the isomeric structure **2a** seemed the most likely one. Synthesis of **2a** by the route shown in eq 1 and comparison of the physical properties of the synthetic compound with those of the acid derived from sparsomycin showed that they were identical.

Mild acid hydrolysis of sparsomycin led to three products.



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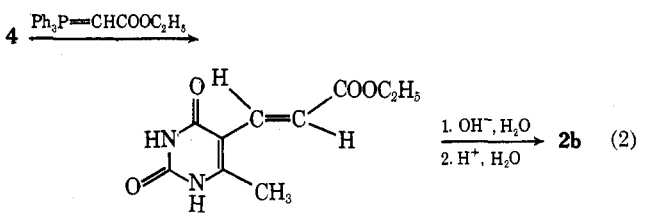
Cooling the reaction mixture gave a crystalline product (**2b**) having the molecular formula $C_8H_8N_2O_4$. Analytical data were consistent with such a formula, but the mass spectrum did not give a molecular ion of 196 as required but gave an *m/e* of 152. Since titration indicated the presence of a carboxyl group, **2b** was converted to a methyl ester which gave the mass spectrum molecular weight expected for the methyl ester of a $C_8H_8N_2O_4$ acid. The compound **2b** differs from **2a** by the elements of CH=CH, and its NMR spectrum has a doublet of doublets centered at δ 7.25 (J = 16 Hz) which would be indicative of a trans vinyl system. These data point to a structure represented by the expression **2b.** Synthesis of **2b** by the route shown in eq 2 and comparison of the product with that derived from



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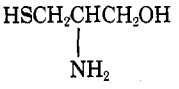
sparsomycin using various physical properties (TLC, ir, uv, NMR, etc.) showed that the two were the same. Alkaline hydrolysis of sparsomycin also formed **2b.** A second product of the acid hydrolysis was isolated only as its diacetyl derivative. Analyses and mass spectra indicated a molecular formula of $C_7H_{13}NSO_3$. Its NMR spectrum showed that it contained two acetyl groups, and the ir spectrum suggested the presence of an acetate and an acetyl derivative of a primary amine. The NMR spectrum of the acetyl derivative and subsequently discussed degradative studies suggested that the compound formed from sparsomycin contained a three-carbon chain with each carbon being substituted by one of the elements, nitrogen, oxygen, or sulfur. Cysteinol (**6**) would be such a compound. The triacetyl derivative of L-cysteinol was reported



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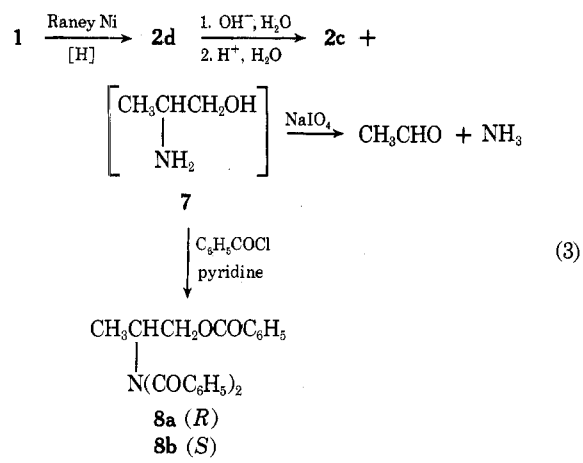
by Enz and Cecchinato[4] to have been prepared by reduction of cysteine ethyl ester followed by acetylation. If the product isolated from sparsomycin is a derivative of either D- or L-

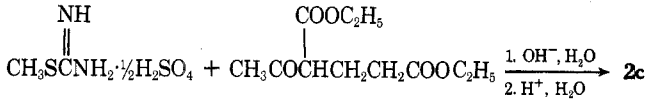
cysteinol, it would be expected to give a triacetyl derivative as was reported previously. However, as the only evidence presented by Enz and Cecchinato for triacetylation was analysis, and since their product gave the same melting point as the compound reported here, it may be that a diacetyl derivative was the one actually prepared previously. In any case it is probable that cysteinol is formed by acid hydrolysis of sparsomycin. The third product was formaldehyde which was identified as its 2,4-dinitrophenylhydrazone.

Treatment of sparsomycin with Raney nickel in boiling ethanol in order to bring about desulfurization gave two products, a gas and a solid (**2d**). The gaseous product was shown by its ir spectrum to be methane which was formed in the ratio of 2 mol to 1 mol of starting material. The high-resolution mass spectrum of the solid product indicated its molecular formula to be $C_{11}H_{17}N_3O_4$ although it was not obtained sufficiently pure to give good analytical values. Alkaline hydrolysis of **2d** followed by acidification formed an acid (**2c**) which had the molecular formula $C_8H_{10}N_2O_4$. In addition to **2c** the hydrolyzed material formed a second product which was isolated only as its tribenzoyl derivative (**8a**). That **8a** contained three benzoyl residues was shown by its mass spectrum. When the alkaline hydrolysate was neutralized followed by periodate oxidation, the products were acetaldehyde and ammonia (eq 3). The former was isolated and



identified as its 2,4-dinitrophenylhydrazone and the latter as its salt with *p*-hydroxyazobenzene-*p'*-sulfonic acid.

The fact that **2c** had the molecular formula of **2b** with two atoms of hydrogen added, and that it was isolated from a reaction involving the use of Raney nickel, suggested that **2c** was the compound resulting from reduction of the olefinic group in **2b.** That this was the case was established by the synthesis of **2c** using the procedure used by Johnson and Heyl[5] (eq 4) to synthesize 1,2,3,4-tetrahydro-6-methyl-2,4-dioxo-5-pyrimidineacetic acid and comparing the product derived from sparsomycin with the synthetic compound.



(4)

In view of the structure of **2c** it seemed probable that **8a** was a derivative of the compound which was oxidized by periodate to give acetaldehyde and ammonia. If it is assumed that hydrolysis of **2d** occurs in a straightforward fashion with addition of only one molecule of water to give the two products, then the compound formed in addition to **2c** would have the molecular formula C_3H_9NO. It would then be, since it is oxidized by periodate to give acetaldehyde and ammonia, either 2-aminopropanol or 2-hydroxypropylamine. Synthesis of the tribenzoyl derivative (**8b**) of (*S*)-2-aminopropanol gave a compound which was identical in most properties (melting point, ir, NMR, R_f in TLC) with **8a** but had a rotation of +63.2° as compared to −61.4° for **8a** in the same solvent. Consequently **8a** must be the tribenzoyl derivative of (*R*)-2-aminopropanol (**7**) which would be expected to react with periodate to give acetaldehyde and ammonia.

The isolation of **2b** by hydrolysis of sparsomycin suggests that **2b** is combined through its carboxyl group in an ester or amide linkage with a $C_5H_{12}NO_2S$ moiety. Since sparsomycin is not basic, the attachment must be through the amino nitrogen present in the (*R*)-2-aminopropanol formed as shown in eq 4. In such case the acid **2c** must be combined with (*R*)-2-aminopropanol as the structure indicated in the expression **2d.** The NMR spectrum of sparsomycin contains two singlet signals attributable to methyl groups. The CH_3C of **7** then cannot be present in sparsomycin as its NMR spectrum would have a doublet arising from a methyl group. This group then must be formed in the desulfurization, and the carbon atom of its methyl group must be attached to a sulfur atom which is part of a $C_2H_5S_2O$ moiety. The evolution of 2 mol of methane during desulfurization indicates that this moiety must have an –S–C–S–C arrangement with a terminal methyl. The NMR spectrum of sparsomycin has signals attributable to three methylene groups. One of these is a complex AB pattern (of an ABX) system with signals centered at δ 3.65 and must be the methylene of a hydroxymethyl group as addition of an acylating agent to the NMR solution causes a downfield shift of these signals. Another AB complex centered at δ 3.14 shows the presence of an adjacent hydrogen atom and so must be the methylene group which is attached to sulfur but which becomes a methyl group on desulfurization. A third methylene group generates a doublet of doublets (δ 3.85 and 4.02) which must arise from a methylene having no adjacent hydrogen atoms so it must be attached to the two sulfur atoms. Oxidation of sparsomycin with hydrogen peroxide forms a product which was shown by analysis, ir spectrum, mass spectrum, and hydrolysis to **2b** to be the derivative of sparsomycin produced by oxidation of the two sulfur atoms to sulfones with no other change. This compound has a resonance in its NMR spectrum at δ 3.26 appropriate for CH_3SO_2,[6] but the signal present in sparsomycin's NMR spectrum at δ 2.30 is absent thus showing that the δ 2.30 resonance can be assigned to a CH_3S group. The presence of a $C_2H_5S_2O$ moiety containing CH_3SCH_2 suggests that in sparsomycin the group $CH_3SCH_2S{=}O$ is present, and this is confirmed by two facts. The acid hydrolysis of sparsomycin gives, among other products, formaldehyde, which would be expected as a product based on the work of Ogura and Tsuchihashi[7] if the above group were present. Furthermore, the chemical shift of δ 3.14 due to a methylene between H_2NCH and S would be more expected if the sulfur were present as sulfoxide.[6] The stereochemistry of the sulfoxide group was not established. The sum of these arguments leaves very little doubt that the structure of sparsomycin is as shown in the expression **1** although it is not intended that there be any suggestion as to the chirality of the sulfoxide group.

Irradiation of an aqueous solution of sparsomycin with an ordinary 15-W fluorescent desk lamp causes isomerization to isosparsomycin. Following the conversion by decrease in the ultraviolet peak at 302 nm led to the conclusion that about 60% of the material was present as isosparsomycin. Analysis indicated that the new product was an isomer as did its NMR spectrum which was almost identical with that of sparsomycin except for the signals due to olefinic hydrogen atoms. These signals in sparsomycin were a doublet of doublets centered at δ 7.24 and 7.45 with J = 16 Hz. In the isomeric compound the olefinic doublets have chemical shifts of δ 6.14 and 6.47 with J = 12 Hz. The decrease in the coupling constant shows that the conversion of sparsomycin to its isomer consists in isomerization of the trans olefin to a cis olefin.[8]

Experimental Section

Melting points are corrected.

Anhydrous Sparsomycin (1). A sample of sparsomycin prepared and dried as was done for the original analyses[2] contained 3.48% water. A sample of this composition was dried for 72 h at room temperature at 0.01 mm and analyzed.

Anal. Calcd for $C_{13}H_{19}N_3O_5S_2$: C, 43.21; H, 5.30; N, 11.63; S, 17.76; O, 22.14. Found: C, 43.32; H, 5.68; N, 11.41; S, 17.48; O, 22.11.

1,2,3,4-Tetrahydro-2,4-dioxo-6-methyl-5-pyrimidinecarboxylic Acid (2a) from Sparsomycin. A 1% aqueous solution of $KMnO_4$ was added dropwise to a suspension of 1 g of sparsomycin in 100 ml of water until a pink color persisted. About 250 ml of solution was required. A little ethanol was added to destroy excess $KMnO_4$, and the solution was filtered through diatomaceous earth. The filtrate was evaporated to dryness under reduced pressure, and the residue was dissolved in 100 ml of water. The resulting solution was stirred for about 2 h with 50 ml of Amberlite IR-120 (H^+). The supernatant was removed by decantation, and the resin was washed thoroughly with water. The combined supernatant and washings was filtered through diatomaceous earth, and the filtrate was concentrated under reduced pressure to a volume of about 5 ml. Refrigeration gave 236 mg. One charcoal treatment and two recrystallizations from water gave 119 mg: mp 242 °C dec; ir (Nujol) 3360, 3280, 3120, 1720, 1620, 1575, and 1515 cm^{-1}; uv (H_2O) max 267 nm (ϵ 8225), (0.1 N HCl) max 222 nm (ϵ 11 100), 272 (10 850), (0.1 N NaOH) max 288 nm (ϵ 6800); NMR (DMF-d_7) δ 2.70 (s, 3 H, CH_3), 12–13 (broad peak, 2–3 H, exchangeable); mass spectrum m/e 170 (M^+).

Anal. Calcd for $C_6H_6N_2O_4$: C, 42.36; H, 3.56; N, 16.47. Found: C, 42.49; H, 3.59; N, 16.47.

5-Hydroxymethyl-6-methyluracil (3). This was synthesized by a slight modification of Kircher's[9] procedure in which condensation of formaldehyde with 6-methyluracil was carried out in base. The sodium salt formed was dissolved in water and neutralized with the theoretical amount of acetic acid. The overall yield was 43%. It was found that Kircher's procedure was highly erratic and his yields could not be duplicated.

1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinecarboxaldehyde (4). A solution of 936 mg (6 mmol) of 5-hydroxymethyl-6-methyluracil in 80 ml of acetic acid was stirred while adding dropwise a solution of 400 mg (4 mmol) of CrO_3 in 40 ml of 50% acetic acid. The solution was then stirred at room temperature for 4 h followed by evaporation to dryness under reduced pressure at 35 °C. The residue was dissolved in 20 ml of water, and the solution was mixed thoroughly with 8 g of silica gel. The mixture was allowed to dry in a current of air with frequent stirring. The dried powder was added to a column containing 50 g of silica gel packed in a mixture of methyl ethyl ketone–acetone–water (7:2:1). The column was then eluted with the same solvent system collecting 103 5-ml fractions. Fractions 21–32 were combined on the basis of weight analysis and TLC on silica gel plates using the above solvents in the ratio 70:20:11. Evaporation of the pooled fractions under reduced pressure gave 378 mg of residue. Recrystallization from water gave 189 mg, mp >200 °C dec: R_f 0.60 (above system); ir (Nujol) 1740 cm^{-1} (CHO); uv (H_2O) max 231 nm (ϵ 6440), 283 (9500); NMR (DMF-d_7) δ 2.54 (s, 3 H, CH_3), 9.98 (s, 1 H, CHO); mass spectrum m/e 154 (M^+).

Anal. Calcd for $C_6H_6N_2O_3$: C, 46.76; H, 3.92; N, 18.18. Found: C, 47.16; H, 4.34; N, 17.87.

1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinecarboxylic Acid (2a). From 4. A mixture of 154 mg (1 mmol) of 4 and 10 ml of 0.1 N NaOH was stirred while slowly adding a solution of 106 mg (0.06 mmol) of $KMnO_4$ in 10.6 ml of water. A little ethanol was added to discharge the color, and the solution was filtered through diatomaceous earth. The filtrate was adjusted to pH 2.5 with 1 N HCl and evaporated to dryness under reduced pressure. The residue was triturated with 1.5 ml of water, and the mixture was filtered. The filter cake was washed with water and recrystallized from water after charcoal treatment: yield 28 mg (16%); mp 246 °C dec; ir and uv spectra identical with those of material isolated from sparsomycin; mass spectrum m/e 170.0286 (calcd for $C_6H_6N_2O_4$, 170.0323).

Anal. Calcd for $C_6H_6N_2O_4$: C, 42.36; H, 3.50; N, 16.47. Found: C, 42.50; H, 3.61; N, 16.32.

β-[(*E*)-1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidine]acrylic Acid (2b). A. Acid Hydrolysis of Sparsomycin. A solution of 2 g of sparsomycin in 40 ml of 2 N HCl was heated on the steam bath for 2 h. The residue was diluted to 35 ml with water and allowed to stand for 3 days at room temperature. Filtration gave 370 mg (35%) of solid, mp 255–259 °C dec. Recrystallization twice from water, four times from DMF, and once from water gave 23 mg: mp 265 °C dec; R_f 0.32 (silica gel, EtOH–MeOH–H_2O, 50:45:5); pK_a' (DMF–60% EtOH), 7.90, 11.35; ir (Nujol) 3400, 3300, 3050, 1710, 1620, 1295, 1190, 1090, 1030, 985, 875, 830, and 780 cm^{-1}; uv (H_2O) max 293 nm (ϵ 14 210), sh 270 nm (ϵ 12 740); NMR (DMF-d_7) δ 2.40 (s, 3 H, CH_3C), 7.25 (d of d, 2 H, J = 16 Hz, trans HC=CH), 10.0–11.6 (broad, exchangeable H); mass spectrum m/e 152, 108, 81, 80, 44, 42.

Anal. Calcd. for $C_8H_8N_2O_4$: C, 48.98; H, 4.13; N, 14.28. Found: C, 48.68; H, 4.77; N, 14.11.

B. Basic Hydrolysis of Sparsomycin. A solution of 200 mg of sparsomycin in 20 ml of 1.0 N NaOH was heated under reflux for 8 h. The solution was adjusted to pH 3.0 with 1.0 N HCl and refrigerated for several days. The solid which separated was removed by filtration, wt 65 mg, mp 258–262 °C dec. The ir spectrum and the TLC R_f using the above system were identical with those of **2b** obtained by acid hydrolysis.

C. Synthesis. A mixture of 308 mg (2 mmol) of 4 and 1.39 g (4 mmol) of carbethoxymethylidenetriphenylphosphorane in 20 ml of dry Me_2SO was heated on the steam bath with protection from moisture for 6 h. Most of the Me_2SO was removed by evaporation under reduced pressure. The residue was diluted with 30 ml of water, and the solid precipitate was removed by filtration and recrystallized twice from EtOH: yield 68 mg (15%); mp 299–302 °C dec; R_f 0.41 (silica gel, Skellysolve B–acetone, 1:1); ir (Nujol) 3330, 1710, 1660, and 1620 cm^{-1}; uv (EtOH) max 303 nm (ϵ 17 875), sh 270 nm (ϵ 9400); NMR (Me_2SO-d_6) δ 1.20 (t, 3 H, CH_3CH_2), 2.28 (s, 3 H, CH_3C=), 4.13 (q, 2 H, CH_3CH_2), 7.13 (d of d, 2 H, trans HC=CH), 11.27 (s, 2 H, exchangeable), mass spectrum m/e 224 (M^+).

Anal. Calcd for $C_{10}H_{12}N_2O_4$: C, 53.57; H, 5.39; N, 12.50. Found: C, 53.93; H, 5.29; N, 13.08.

A solution of 300 mg of the above material (5) in 20 ml of 1 N NaOH was boiled for 2 h. The solution was acidified with excess concentrated HCl, allowed to stand for a few hours, and filtered, yield 240 mg. Recrystallization from water gave 151 mg (58%), mp 271 °C dec; the ir, uv, and NMR spectra of this product were essentially identical with those of the acid derived from hydrolysis of sparsomycin. The R_f values on silica gel TLC plates using methyl ethyl ketone–acetone–H_2O (70:20:11), EtOH–MeOH–H_2O (50:45:5), and MeOH–H_2O (9:1) were identical and were respectively 0.13, 0.40, and 0.75.

Anal. Calcd. for $C_8H_8N_2O_4$: C, 48.98; H, 4.13; N, 14.28. Found: C, 48.84; H, 4.52; N, 14.13.

Methyl β-[(*E*)-[1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidine]acrylate. A mixture of 195 mg of **2b** and 20 ml of methanol was cooled in an ice bath while saturating with dry HCl. The mixture was allowed to stand at room temperature overnight. The solvent was removed by evaporation under reduced pressure, and the residue was repeatedly evaporated under reduced pressure with methanol. The product was recrystallized three times from methanol: yield 55 mg; mp 305–307 °C dec; ir (Nujol) 3330, 1720, 1660, and 1620 cm^{-1}; NMR (DMF-d_7) δ 2.40 (s, 3 H, CH_3C), 3.69 (s, 3 H, CH_3O), 7.28 (d of d, 2 H, J = 16 Hz, trans HC=CH), 11.15–11.34 (broad, 2 H, exchangeable); mass spectrum m/e 210.0676 (calcd for $C_9H_{10}N_2O_4$, 210.0644).

Anal. Calcd for $C_9H_{10}N_2O_4$: C, 51.42; H, 4.80; N, 13.20. Found: C, 51.22; H, 5.17; N, 13.35.

Di-*O,N*-Acetylcysteinol. One gram of sparsomycin was hydrolyzed as previously described for acid hydrolysis. After removal of **2b** the filtrate was evaporated to dryness under reduced pressure, and the residue was repeatedly evaporated under reduced pressure with methanol. The 554 mg of residue was mixed with 10 ml of dry pyridine and 2 ml of acetic anhydride, and the mixture was stirred overnight. Two milliliters of methanol was added, and the solution was evaporated to dryness under reduced pressure with frequent additions of methanol. The residue was mixed with 20 ml of water, and the mixture was extracted with four 20-ml portions of $CHCl_3$. The combined extracts were washed with two 10-ml portions of 0.1 N HCl and two 10-ml portions of water followed by drying ($MgSO_4$), filtration, and evaporation under reduced pressure. The residue (212 mg) was chromatographed on 10 g of silica gel using $CHCl_3$ until 100 5-ml fractions had been collected. The column was then developed with $CHCl_3$–MeOH (98:2) until a second 100 5-ml fractions had been collected. Fractions 28–50 of the second 100 were combined on the basis of a weight analysis and evaporated under reduced pressure, yield 110 mg. A portion of this was recrystallized from benzene: mp 99–100 °C; R_f 0.19 (silica gel, cyclohexane–EtOAc–95% EtOH, 5:3:2); ir (Nujol) 3230, 1740, 1645, and 1545 cm^{-1}; NMR ($CDCl_3$) δ 1.97 (s, 3 H, CH_3CO), 2.05 (s, 3 H, CH_3CO), 2.82–3.08 (m, 2 H, CH_2S), 4.15–4.65 (m, 3 H, OCH_2CHN), 6.73 (d, 1 H, NH).

Anal. Calcd for $C_7H_{13}NSO_3$: C, 43.98; H, 6.85; N, 7.32; S, 16.78. Found: C, 44.05; H, 6.70; N, 7.06; S, 16.54.

Formaldehyde from Sparsomycin. Two grams of sparsomycin was hydrolyzed with acid as already described. After **2b** had been

removed, the filtrate was extracted with 20 ml of benzene and three 20-ml portions of ether. One-fourth of the remaining aqueous phase was diluted with 300 ml of Brady's reagent. After the mixture had stood at room temperature for 3 days, it was filtered to give 103 mg of solid, mp 163–165 °C. Recrystallization from alcohol did not change the melting point. A mixture melting point with authentic formaldehyde 2,4-dinitrophenylhydrazone was not depressed, and the two compounds had identical ir spectra.

β-[(*R*)-*N*-(2-Hydroxy-1-methylethyl)-1,2,3,4-tetrahydro-6-methyl-2,4-dioxo-5-pyrimidine]propionic Acid (2d). A mixture of 400 mg of sparsomycin, 8 g of Raney nickel, and 200 ml of water was boiled and stirred for 21 h. The Raney nickel was removed by filtration and was washed with two 50-ml portions of boiling water. The combined filtrate and washings were evaporated to dryness under reduced pressure. The residue was dissolved in 50 ml of methanol, and the solution was filtered through diatomaceous earth. The filtrate was concentrated under reduced pressure, and the residue (188 mg) was recrystallized from methanol: yield 32 mg; mp 231 °C; mass spectrum *m/e* 255.1223 (calcd for $C_{11}H_{17}N_3O_4$, 255.1219).

Anal. Calcd for $C_{11}H_{17}N_3O_4$: C, 51.76; H, 6.71; N, 16.46. Found: C, 50.89; H, 5.47; N, 16.20.

Methane from Sparsomycin. A mixture of 16 g of Raney nickel and 400 ml of water was stirred and heated to boiling. Sparsomycin (760 mg, 2 mmol) was added. The gas which evolved was collected. There was obtained 105 ml (theoretical for 4 mmol 98.8 ml) of gas identified by its ir spectrum as methane containing a little carbon dioxide.

β-(1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidine)-propionic Acid (2c). From 2d. Crude **2d** (600 mg) was dissolved in 40 ml of 1.0 N NaOH, and the solution was boiled for 8 h. After the reaction mixture had cooled, it was filtered. The filtrate was adjusted to pH 3.5 with concentrated HCl and concentrated under reduced pressure to 3–4 ml. Refrigeration gave 230 mg. Charcoal treatment and several recrystallizations from water gave 99 mg: mp 302–304 °C dec; ir (Nujol) 3050, 1720, 1630, 1530, 1375, 1340, 1280, 1210, 1175, 870, 815, 792, and 775 cm^{-1}; uv (H_2O) max 266 nm (ε 8237); NMR (Me_2SO-d_6) δ 2.08 (s, 3 H, CH_3C), 2.26–2.60 (m, 4 H, CH_2CH_2), 10.63 (s, 1 H, exchangeable), 10.91 (s, 1 H, exchangeable); mass spectrum *m/e* 198.0646 (calcd for $C_8H_{10}N_2O_4$, 198.0640).

Anal. Calcd for $C_8H_{10}N_2O_4$: C, 48.49; H, 5.09; N, 14.14. Found: C, 48.51; H, 5.17; N, 14.14.

Ethyl β-(2-Methylmercapto-6-methyl-4-oxo-5-pyrimidine)propionate. *S*-Methylisothiourea sulfate (21 g, 0.075 mol) was dissolved in 225 ml of water. Diethyl α-acetylglutarate (11.5 g, 0.15 mol) and 16.8 g (0.3 mol) of potassium hydroxide were added. The mixture was stirred overnight at room temperature and heated for 1 h on the steam bath. The mixture was refrigerated, and the supernatant was decanted. The residue was crystallized from EtOH, yield 1.7 g, mp 168 °C. Two recrystallizations from EtOH did not change the melting point: uv (EtOH) max 235 nm (ε 9216), 288 (8450); NMR (Me_2SO-d_6) δ 1.18 (t, 3 H, CH_3C), 2.22 (s, 3 H, CH_3C), 2.37–2.62 (m, 7 H, CH_3S and CH_2CH_2), 4.06 (q, 2 H, OCH_2CH_3), 11.66–12.81 (broad, 1 H, NH).

Anal. Calcd for $C_{11}H_{16}N_2SO_3$: C, 51.57; H, 6.30; N, 10.93; S, 12.54. Found: C, 51.72; H, 6.38; N, 11.11; S, 12.48.

1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinepropionic Acid (2c). Synthesis. A mixture of 2.5 g of ethyl β-(2-methylmercapto-6-methyl-4-oxo-5-pyrimidine)propionate and 50 ml of concentrated HCl was boiled for 8 h. Refrigeration gave 1.55 g, mp 295–297 °C dec. Recrystallization from water and DMF gave mp 303–305 °C dec; ir and NMR spectra were identical with those of **2c** from **2d**; mass spectrum *m/e* 198 (M^+).

Anal. Calcd for $C_8H_{10}N_2O_4$: C, 48.49; H, 5.09; N, 14.14. Found: C, 48.54; H, 5.24; N, 14.30.

Tribenzoyl-(*R*)-alaninol (8a). The filtrate from the hydrolysis of **2d** to give **2c** was freeze dried. A mixture of the residue with 20 ml of dry pyridine and 2 ml of benzoyl chloride was heated for 2 h on the steam bath. The pyridine was removed by evaporation under reduced pressure, and the residue was partitioned between 10 ml of $CHCl_3$ and 20 ml of water. The aqueous layer was extracted with two 10-ml portions of $CHCl_3$. The combined $CHCl_3$ layers were washed with 10 ml of 1 N HCl, 10 ml of saturated $NaHCO_3$ solution, and two 10-ml portions of water and dried ($MgSO_4$). After the solution was filtered and evaporated to dryness under reduced pressure, the residue (1.17 g) was chromatographed on 60 g of silica gel using $CHCl_3$ as the eluent. After 150 5-ml fractions were collected, fractions 65–82 were combined on the basis of a weight analysis and TLC (R_f 0.18, silica gel, $CHCl_3$). Evaporation of the pool under reduced pressure gave 282 mg which was recrystallized twice from EtOH: yield 86 mg; mp 87–89 °C; R_f 0.75 (silica gel, $CHCl_3$–MeOH, 95:5) identical with that of **8b**; $[\alpha]_D$ −61.4° (*c* 1, $CHCl_3$); ir (Nujol) 1720 (ester CO), 1660 (amide CO), 1595, and 1575 cm^{-1}; NMR ($CDCl_3$) δ 1.52 (d, 3 H, CH_3), 4.40–5.40 (m, 3 H, CH_2CH), 6.9–8.0 (m, 15 H, aromatic); mass spectrum *m/e* low resolution 387, 330, 265, 252, 208, 160, 105, 77, high resolution 387.1483 (calcd for $C_{24}H_{21}NO_4$, 387.1470).

Tribenzoyl-(*S*)-alaninol (8b). This was done essentially as was the preparation of **8a** but using 3 g of (*S*)-alaninol, 15 ml of benzoyl chloride, 150 ml of dry pyridine, and proportional amounts of other materials. Chromatography was done on 550 g of silica gel and 437 20-ml fractions were collected. Fractions 201–310 were combined and evaporated to dryness under reduced pressure, yield 9.6 g. Four recrystallizations from EtOH gave 2.6 g: mp 90–91 °C; TLC (see **8a** preparations); $[\alpha]_D$ +63.2° (*c* 2, $CHCl_3$); ir, NMR, and mass spectrum were the same as for tribenzoyl-(*R*)-2-aminopropanol (**8a**); mass spectrum *m/e* 387.1495 (calcd for $C_{24}H_{21}NO_4$, 387.1470).

Anal. Calcd for $C_{24}H_{21}NO_4$: C, 74.40; H, 5.46; N, 3.62. Found: C, 74.35; H, 5.83; N, 3.93.

Periodate Oxidation of 2d Hydrolysate. After removal of **2c** from the hydrolysate of **2d** derived from 200 mg of sparsomycin, sodium periodate (0.43 g, 2 mmol) was added, and the solution was allowed to stand overnight. Nitrogen was bubbled through the reaction mixture and then through 200 ml of Brady's reagent for 4 h. Filtration gave 14 mg of yellow solid, mp 148–150 °C. A mixture melting point with authentic acetaldehyde 2,4-dinitrophenylhydrazone (mp 152–154 °C) was 148–150 °C while a mixture melting point with the propionaldehyde derivative (mp 149–151 °C) was 131–136 °C.

The aqueous residue after removal of acetaldehyde was adjusted to pH 10 with NaOH solution. The solution was steam distilled into 10 ml of 0.1 N HCl until no more volatile base was distilled. Filtration indicated that 0.3 mmol of volatile base had been distilled. The solution was again made strongly basic with NaOH solution and steam distilled into a solution of 140 mg of *p*-hydroxyazobenzene-*p'*-sulfonic acid in 25 ml of water. The solution was evaporated to dryness under reduced pressure, and the residue was recrystallized twice from water. The ir spectrum of the product was identical with that of ammonium *p*-hydroxyazobenzene-*p'*-sulfonate.

Peroxide Oxidation of Sparsomycin. One gram of sparsomycin was dissolved in 90 ml of acetic acid and 10 ml of 30% H_2O_2 was added. After the solution had stood at room temperature for 1 day, it was evaporated to dryness at room temperature under high vacuum. The residue was triturated with water and again evaporated to dryness under reduced pressure. This procedure was repeated twice. One recrystallization from water gave 680 mg of which 180 mg was again recrystallized from water to give 102 mg: mp 251 °C dec; $[\alpha]_D$ +45° (*c* 0.5, H_2O); ir (Nujol) 3500, 3210, 1720, 1660, 1585, 1300, 1225, 1155, 1135, 1055, 1022, 975, 865, and 783 cm^{-1}; uv (H_2O) max 301 nm (ε 22 600) sh 270 (14 050); NMR (Me_2SO-d_6) δ 2.24 (s, 3 H, CH_3C), 3.25 (s, 3 H, CH_3SO_2); mass spectrum *m/e* 409 (calcd, 409).

Anal. Calcd for $C_{13}H_{19}N_3S_2O_8$: C, 38.12; H, 4.68; N, 10.26; S, 15.66; O, 31.26. Found (corrected for 3.18% water content): C, 38.40; H, 4.96; N, 10.13; S, 15.58; O, 33.08.

An acid hydrolysis of 500 mg of the peroxide product using the same procedure as was used to convert sparsomycin to **2b** gave 90 mg of material which had the same R_f in TLC in EtOH–MeOH–H_2O (50:45:5) as **2b**. Recrystallization from water with charcoaling gave 32 mg of product which had the same NMR spectrum as **2b** and the same R_f values in TLC as **2b** in the system already mentioned.

Isosparsomycin. A solution of 500 mg of sparsomycin in 750 ml of water was irradiated with a fluorescent desk lamp for 7 days. The solution was evaporated to dryness under reduced pressure. The residue was subjected to countercurrent distribution in a 10 ml per phase machine using the system ethyl acetate–butanol–water (3:7:10) for 400 transfers. Tubes 46–80 (K = 0.19) were pooled and evaporated to dryness under reduced pressure giving 247 mg of amorphous residue. The residue was dissolved in 5 ml of hot water, and the solution was filtered through diatomaceous earth. Slow evaporation of the filtrate at room temperature gave a crystalline precipitate. Recrystallization from water gave 91 mg: mp 158–164 °C; R_f 0.16 (silica gel; *n*-BuOH–EtOH–H_2O, 70:27:3); ir (Nujol) 3375, 3325 (sh), 1745, 1710, 1675, 1640, 1555, 1525 (sh), 1445, 1425, 1370, 1310, 1270, 1245, 1098, 1065, 1018, 1002, 975, 955, 895, 750, and 725 cm^{-1}; NMR (DMF-d_7) δ 2.11 (s, 3 H, CH_3C), 2.30 (s, 3, CH_3S), 3.10 (m, 2 H, $CHCH_2SO$), 3.65 (m, 2 H, CH_2O), 3.95 (d of d, 2 H, $SOCH_2S$), 4.35 (m, 1 H, CHNH), 6.31 (d of d, 2 H, J = 12 Hz, cis HC=CH).

Anal. Calcd for $C_{13}H_{19}N_3O_6S_2$: C, 41.37; H, 5.08; N, 11.14; S, 16.99. Found: C, 42.09; H, 5.32; N, 11.13; S, 17.28.

Registry No.—**1**, 1404-64-4; **1** peroxide derivative, 58462-94-5; **2a**, 51622-67-4; **2b**, 28277-67-0; **2b** Me ester, 28425-66-3; **2c**, 28181-39-7; **2d**, 28277-69-2; **3**, 147-61-5; **4**, 24048-74-6; **5**, 28277-68-1; **6** *O,N*-di-

acetyl derivative, 58462-95-6; **8a**, 29537-31-3; **8b**, 28277-70-5; ethyl β-(2-mercapto-6-methyl-4-oxo-5-pyrimidine)propionate, 58462-96-7; *S*-methylisothiourea sulfate, 867-44-7; diethyl α-acetylglutarate, 1501-06-0; benzoyl chloride, 98-88-4; (*S*)-alaninol, 2749-11-3; isosparsomycin, 58462-97-8.

References and Notes

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(2) A. D. Argoudelis and R. R. Herr, *Antimicrob. Ag. Chemother.*, 780 (1962).
(3) P. F. Wiley and F. A. MacKellar, *J. Am. Chem. Soc.*, **92**, 417 (1970).
(4) W. Enz and M. Cecchinato, *Helv. Chim. Acta*, **44**, 706 (1961).
(5) T. B. Johnson and F. W. Heyl, *Am. Chem. J.*, **38**, 659 (1907).
(6) F. A. Bovey, "NMR Data Tables for Organic Compounds", Vol. 1, Interscience, New York, N.Y., 1967, pp 152, 217–219.
(7) (a) K. Ogura and G. Tsuchihashi, *Chem. Commun.*, 1689 (1970); (b) G. Tsuchihashi and K. Ogura, *Bull. Chem. Soc. Jpn.*, **44**, 1726 (1971); (c) K. Ogura and G. Tsuchihashi, *Tetrahedron Lett.*, 1383 (1972).
(8) L. M. Jackman, "Nuclear Magnetic Resonance Spectroscopy", Pergamon Press, Elmsford, N.Y., 1959, p 85.
(9) W. Kircher, *Justus Liebigs Ann. Chem.*, **385**, 293 (1911).

Sigmatropic Hydrogen Migration and Electrocyclization Processes in Compounds in the Vitamin A Series. Photochemistry of Polyenes. 10[1]

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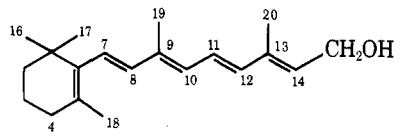
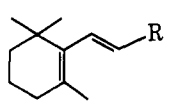
Reactions of dienes, trienes, and tetraenes in the vitamin A series brought about by direct irradiation were examined. In addition to the previously known sigmatropic 1,5-hydrogen migration from C-18 to C-8 and the hitherto unnoticed electrocyclization, geometric isomerization also appears to be an important reaction from S_1. Electrocyclization and geometric isomerization are reversible processes; hence in most cases the end products are retro-γ derivatives. Mechanistically it was shown that the hydrogen migration process can originate from either the 7-cis or 7-trans isomers of the conjugated systems. A case of 6e-electrocyclic ring opening process involving both the excited states of the product and the reactant is presented.

Compounds in the vitamin A (I) series are known to undergo a variety of photochemical reactions. In addition to the geometric isomerization reactions, which appear to be the exclusive reaction of the triplet states,[3] direct irradiation leads to hydrogen migration, cyclization, and intramolecular cycloaddition products. In this paper, the sigmatropic 1,5-hydrogen migration and 6e-electrocyclization processes are examined.

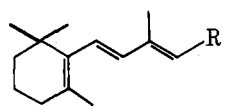


I



II

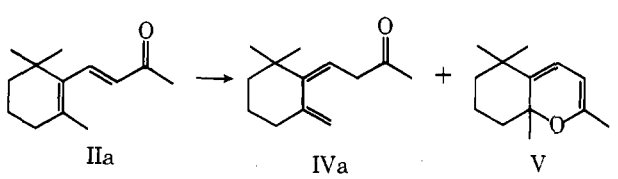
a, R = $COCH_3$
b, R = $CHCH_3OH$
c, R = CN



III

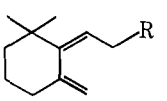
a, R = CN
b, R = CO_2Et
c, R = CO_2H
d, R = H
e, R = CHO
f, R = $COCH_3$

The earliest report on hydrogen migration in compounds in this series was on β-ionone (IIa) in 1957,[4] but its correct structure, a retro-γ derivative (IVa), was not recognized until 4 years later.[5] In this case, it is a minor product, the major being the α-pyran V which was later shown to be in equilibrium with *cis*-ionone.[6] In a series of papers in the early 1960's, Mousseron-Canet and co-workers[7] established the generality of the sigmatropic hydrogen migration reaction in trienes (β-ionylidene derivatives, III) as well as dienes in the vitamin A series. Furthermore, because of detection of 7-cis isomer(s) prior to significant accumulation of the retro-γ products, they suggested that the retro-γ products are formed by way of the 7-cis isomer(s) in two separate photochemical steps.



IIa IVa V

The initially formed retro-γ products are believed to have the *Z* configuration. This was deduced from their lack of reactivity toward maleic anhydride.[7a] More recently, it was found that (*Z*)-retro-γ-ionone undergoes secondary photochemical reactions of geometric isomerization and internal cycloaddition giving (*E*)-retro-γ-ionone and the tricyclic ether shown.[8] Upon heating, (*E*)-retro-γ-ionone [(*E*)-IVa] further rearranged to a cyclobutene[8]—one of few cases where a cyclobutene is more stable than the corresponding diene.[9]



(*Z*)-IV

a, R = $COCH_3$
b, R = $CHCH_3OH$
c, R = CN