tracts were dried, filtered, and evaporated to yield 400 mg of an oil; λ_{max}^{EtOH} 250 mμ; homogeneous by tlc.

Chromic Acid Oxidation of β-Dimethylaminopropiophenone (XII). The amino ketone XII (5.0 mmoles) was mixed with 10 ml of trifluoroacetic acid, and to the solution was slowly added over a period of 1 hr a mixture prepared from chromium trioxide (2.5 g, 25 mmoles), water (4 ml), and glacial acetic acid (40 ml). After the addition was complete, the reaction mixture was heated at 50° with stirring for 3 hr. The mixture was evaporated at reduced pressure and 50°, leaving a residue which was dissolved in 100 ml of 5% sulfuric acid and then was extracted with five 25-ml portions of ether. The combined extracts were filtered and evaporated, and the addition of bisulfite to an alkaline solution of the residue precipitated chromium salts. After filtration, acidification followed by extraction into ether yielded benzoic acid which was purified by sublimation (290 mg, 48%).

The original aqueous solution was treated with 4 g of sodium bisulfite, adjusted to pH 10, and filtered, and the filtrate was washed with methylene chloride. The aqueous solution was then adjusted to pH 6 and applied to 175 ml of AG 50W-X8, 200–400 mesh, hydrogen form, cation-exchange resin. The column was washed with water until the eluate was neutral and then with 1.5 *N* ammonium hydroxide. The first 100 ml of basic eluate was evaporated at 50° and reduced pressure until the pH of the solution was about 8. The solution was then applied to 100 ml of AG 1-X8, 200–400 mesh, hydroxide form, anion-exchange resin. Again, the column was washed with water until the eluate was neutral and then with 2 *N* hydrochloric acid. The first 100 ml of acidic eluate was evaporated to a semisolid residue, water was added, and the process repeated until all of the excess hydrochloric acid was removed, leaving a crystalline residue of N,N-dimethylglycine (XIV) hydrochloride, 200 mg, 30%. This product was purified by sublimation [115° (10 μ)] to yield material, mp 182–183°.

Anal. Calcd for $C_4H_{10}ClNO_2$: C, 34.4; H, 7.2; N, 10.0. Found: C, 34.7; H, 7.4; N, 10.0.

Lead Tetraacetate Oxidation of Dimethylglycine (XIV) Hydrochloride. Dimethylglycine hydrochloride (100 mg, 0.72 mmole) was mixed with 12 ml of benzene, and lead tetraacetate (450 mg, 1.0 mmole) was added to this heterogeneous mixture. While maintaining a sweep of dry nitrogen through the apparatus and with stirring, the reaction vessel was heated to 55°. The liberated carbon dioxide was swept into a gas washing vessel containing 8 ml of 0.1 *N* carbonate-free sodium hydroxide. Gas evolution was complete after 2 hr, and the carbon dioxide was obtained in the usual manner as barium carbonate, 60 mg, 40%. A parallel reaction, omitting the dimethylglycine, gave less than 1 mg of barium carbonate.

The gas washing vessel was removed and replaced by a distillation take-off, whose tip was immersed in a solution (6 ml) prepared from 5.6 g of 5,5-dimethyl-1,3-cyclohexanedione dissolved in 100 ml of 50% ethanol. Water (5 ml) was added to the benzene residue, and while maintaining a slow nitrogen sweep, the mixture was heated to 150–160°. When almost dry, an additional 10 ml of water was added and distillation was continued. This process was repeated twice more, and the total distillate was then evaporated at 50° and reduced pressure after standing at room temperature for 24 hr. The residue was recrystallized several times from 95% ethanol to yield pure formaldehyde dimedone derivative, 100 mg, 48%, mp 192°.

The oxidation vessel was now equipped with a dropping funnel containing 15 ml of 20% sodium hydroxide solution. The distillation take-off was replaced with a gas washing vessel containing 10 ml of 5% hydrochloric acid. While maintaining the nitrogen stream, the alkali was added to the residue in the oxidation vessel which was then heated to 70°. After 2 hr, the acidic wash solution was removed and evaporated at reduced pressure to a residue of 20 mg (34%) of dimethylamine hydrochloride.

The amine salt, 16.2 mg (0.22 mmole), dissolved in 100 μl of water, was added to a solution of *p*-bromobenzenesulfonyl chloride (54 mg, 0.22 mmole) in 80 μl of *p*-dioxane, and 280 μl of 10% aqueous potassium hydroxide was added. The mixture was then shaken for 30 min, an additional 280 μl of the potassium hydroxide solution was added, and the mixture was warmed in a water bath at 55–60° for 2 hr with occasional shaking. The solid was removed, washed with water until the washings were no longer alkaline, dried *in vacuo* at room temperature, and then sublimed [70° (10 μ)] to yield N,N-dimethyl-*p*-bromobenzenesulfonamide, 38 mg, 72%, mp 90–92° (lit.[47] mp 94°).

(47) C. S. Marvel and F. E. Smith, *J. Am. Chem. Soc.*, **45**, 2696 (1923)

Microbiological Hydroxylation of Monocyclic Alcohols

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Abstract: As part of a broad study of the microbiological oxygenation of simple monocyclic systems, cyclododecanol exposed to *Sporotrichum sulfurescens* gave a mixture of dioxygenated products that was oxidized to cyclododecane-1,5-dione, cyclododecane-1,6-dione, and cyclododecane-1,7-dione. The relative yields of the isomeric diones can be rationalized on conformational grounds and suggest a hypothetical enzyme–substrate model in which oxygenation occurs at a methylene group about 5.5 A from the electron-rich substituent of the substrate. Oxygenations of cyclotridecanol to cyclotridecane-1,7-dione and of cyclotetradecanol to cyclotetradecane-1,6-dione were also observed. Cyclohexanol oxygenation *per se* was not seen, but the more lipophilic N-phenylcarbamate was oxygenated to the 4-hydroxy derivative.

The oxygenation of an unactivated methylene group is a reaction at which microorganisms are still more adept than organic chemists, in spite of many advances made in recent years by the macroorganisms. This difference has been widely exploited during the past 15 years to oxygenate microbially a variety of steroids,[1a] and somewhat less widely to oxygenate terpenes,[1b] hydrocarbons,[1c] alkaloids,[1d] and other substances.[1e]

(1) As leading references, see: (a) E. Vischer and A. Wettstein, *Advan. Enzymol.*, **20**, 237 (1958); S. H. Eppstein, P. D. Meister, H. C. Murray, and D. H. Peterson, "Vitamins and Hormones," Vol. XIV, Academic Press Inc., New York, N. Y., 1956, pp 359–432; O. Hanc, "Global Impacts of Applied Microbiology," M. P. Starr, Ed., John Wiley and Sons, Inc., New York, N. Y., 1963, p 420; (b) P. K. Bhattacharyya, B. R. Prema, B. D. Kulkarni, and S. K. Pradhan, *Nature*, **187**, 689 (1960); P. J. Chapman, G. Meerman, I. C. Gunsalus, R. Srinivasan, and K. L. Rinehart, Jr., *J. Am. Chem. Soc.*, **88**, 618 (1966); (c) G. W. Fuhs, *Arch. Mikrobiol.*, **39**, 374 (1961); (d) E. Meyers and S. C. Pan, *J. Bacteriol.*, **81**, 504 (1961); G. D. Griffith, R. U. Byerrum, and W. A. Wood, *Proc. Soc. Exptl. Biol. Med.*, **108**, 162 (1961); (e) D. J. Siehr, *J. Am. Chem. Soc.*, **83**, 2401 (1961); V. Prelog and H. E. Smith, *Helv. Chim. Acta*, **42**, 2624 (1959).

During our studies on microbial oxidations of steroids, we were struck by the apparent lack of a rational explanation for the selection by a given microorganism of the particular carbon atom to be oxygenated. In order to make a limited inquiry into the nature of the controlling factors, we have studied the oxygenation of some simple monocyclic systems.

We have observed that cyclododecanol undergoes facile microbiological hydroxylation with a large variety of microorganisms, whereas cyclododecane is not hydroxylated. For example, when cyclododecanol was subjected to the oxygenating activity of *Sporotrichum sulfurescens*, ATCC 7159, there was obtained, by extraction of the fermentation beer, a complex mixture of cyclododecanediones, hydroxycyclododecanones, and cyclododecanediols. As will be shown below, these compounds arose from oxygenation of the cyclododecanol in the 5, 6, and 7 positions. In order to facilitate the isolation of characterizable isomeric products, the entire crude extracted mixture was oxidized with Jones' chromic acid reagent[2] to a mixture of cyclododecane-1,5-dione, cyclododecane-1,6-dione, and cyclododecane-1,7-dione. The diketones were separated by chromatography and crystallization.

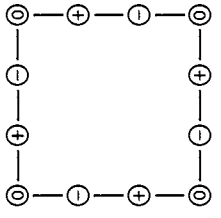
The structure of each dione was established by subjecting it to Baeyer–Villiger oxidation with peroxytrifluoroacetic acid.[3] Alkaline saponification of the resultant lactone mixture gave a neutral fraction containing glycols and an acidic fraction consisting of hydroxy acids and dibasic acids. These cleavage fragments were characterized as such, or by vapor phase chromatography (vpc) of the methyl esters of the dibasic acids derived therefrom by oxidation with Jones' reagent. The degradation of cyclododecane-1,5-dione gave only heptane-1,7-diol and glutaric acid. The absence of other fragments indicates that the preponderant product of the Baeyer–Villiger reaction was 1,7-dioxacyclotetradecane-2,6-dione. Cyclododecane-1,6-dione, which has been characterized previously by Niles and Snyder[4] *via* Beckmann rearrangement of the dioxime, was oxidized to a mixture of all three possible isomeric dilactones. This mixture was hydrolyzed to give hexane-1,6-diol, adipic acid, 7-hydroxyenanthic acid, suberic acid, and 5-hydroxyvaleric acid. Degradation of cyclododecane-1,7-dione also gave rise to both possible isomeric dilactones, of which the symmetrical isomer, 1,8-dioxacyclotetradecane-2,9-dione, could be isolated as a pure crystalline entity. Hydrolysis of this lactone gave only 6-hydroxycaproic acid. Hydrolysis of the lactone mixture gave pentane-1,5-diol and pimelic acid in addition to 6-hydroxycaproic acid.

The oxidative dissimilation of cyclotridecanol and cyclotetradecanol by *Sporotrichum sulfurescens* also resulted in mixtures of diones, diols, and hydroxy ketones which were oxidized to diones. The total yields of products were much lower for cyclotridecanol and cyclotetradecanol than for cyclododecanol. The principal product in the case of cyclotridecanol was cyclotridecane-1,7-dione, which was degraded to pentane-1,5-diol, hexane-1,6-diol, pimelic acid, suberic acid, 6-hydroxycaproic acid, and 7-hydroxyenanthi- acid. The vpc analyses of the derived dimethyl dec carboxylates also revealed the presence of traces of dimethyl succinate and dimethyl pimelate derived from butane-1,4-diol and heptane-1,7-diol that were present in the neutral fraction from the degradation, thus indicating that the purified cyclotridecane-1,7-dione still contained traces of cyclotridecane-1,6-dione. This was substantiated further by the identification of traces of adipic acid (as the dimethyl ester) in the dicarboxylic acid fraction.

The principal product from the bioconversion of cyclotetradecanol was cyclotetradecane-1,6-dione. Degradation of this compound gave octane-1,8-diol, butane-1,4-diol, adipic acid, sebacic acid, and 9-hydroxypelargonic acid. That the purified cyclotetradecane-1,6-dione contained a trace of cyclotetradecane-1,7-dione was shown by the presence of traces of pentane-1,5-diol and heptane-1,7-diol in the neutral fraction, pimelic acid and azelaic acid in the dicarboxylic acid fraction, and 6-hydroxycaproic acid and 8-hydroxycaprylic acid in the hydroxy acid fraction.

For reference purposes, cyclotetradecane-1,7-dione, was readily obtained by catalytic hydration[5] of cyclotetradeca-1,8-diyne.[6] From this reaction the known cyclotetradecane-1,8-dione,[7] was also obtained. These products were formed in approximately equal amounts and were readily separated by chromatography, and the total yield of pure compounds was 80%. Baeyer–Villiger degradation of cyclotetradecane-1,7-dione gave the three possible dilactones, whose saponification afforded the six expected products.

The fact that cyclododecanol undergoes facile microbial hydroxylation, whereas cyclododecane does not, permits the hypothesis that the oxygenating enzyme first attaches itself to the hydroxyl group of cyclododecanol, with subsequent biooxygenation of the substrate. The fact that the oxygenation occurs at positions 5, 6, and 7, and largely at the last two positions, permits the hypothesis that there is a critical spacing for the enzyme attachment–oxygenation reaction site span. Dunitz and Prelog[8] have shown that cyclododecane and certain of its derivatives exist preferentially as "conformer A" in the crystal state. This may be represented two dimensionally as



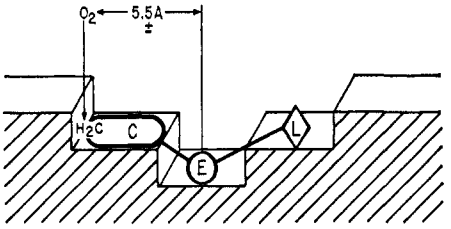
where the "corner" atoms (0) define a plane, with the other atoms falling above (+) or below (−) the plane. Making the assumption that preference for this conformation is retained in solution (which seems probable on the basis of, among other things, spectral data) and, more important, that it is maintained during the

(2) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon *J. Chem. Soc.*, 39 (1946).
(3) W. D. Emmons and G. B. Lucas, *J. Am. Chem. Soc.*, 77, 2287 (1955).
(4) E. T. Niles and H. R. Snyder, *J. Org. Chem.*, 26, 330 (1961).
(5) R. J. Thomas, K. N. Campbell, and G. F. Hennion, *J. Am. Chem. Soc.*, 60, 718 (1938).
(6) J. H. Wotiz, R. F. Adams, and C. G. Parsons, *ibid.*, 83, 373 (1961). We are indebted to Dr. Wotiz of Diamond Alkali Co. for a generous sample of this material.
(7) A. T. Blomquist and R. D. Spencer, *ibid.*, 70, 30 (1948).
(8) J. D. Dunitz and V. Prelog, *Angew. Chem.*, 72, 896 (1960).

enzyme–substrate interaction, then one may measure the several spans from the primary enzyme attachment site [in this case, the hydroxyl group at either position on any one of the three carbon atom types, namely (0), (+), or (−)] to the point of hydroxylative attachment. Measurements of a Dreiding model of cyclododecanol show a large number of approximately 5.5-A spacings from oxygen to the C-6 carbons, a lesser number to the C-7 carbon, and few to the C-5 carbons. This coincides roughly with the relative abundance of the three diketones formed in the bioconversion.

Presumably the observed falling off of reactivity of cyclotridecanol and cyclotetradecanol also has a conformational explanation. The conformations of the larger rings have, unfortunately, not been determined.

In order to suggest additional experiments, we have formulated the following hypothetical enzyme–substrate model based on the experiments cited above



ENZYME

where C represents a suitable cyclic system whose conformation and substitution permit (or induce) a fit to the enzyme region where oxygen is bound or transferred to substrate. E represents an electron-rich group attached to or included in the cyclic system C. L represents a lipophilic group (attached to E), which may be part of the cyclic system C or a separate moiety.

Although this model is presumably applicable to smaller ring size cycloalkanols, we were unable to demonstrate microbial hydroxylation of cyclohexanol, cycloheptanol, or cyclooctanol. Since no substrate was recovered from these experiments, we assumed that these materials were sufficiently volatile to be removed from the fermenter by the air stream used to aerate the fermentation, or that the initial biooxygenation product(s) was readily degraded by the microorganism to small nutrient molecules, or that the products were not readily recoverable from the fermentation medium, or that these cycloalkanols were insufficiently lipophilic to act as substrates. The hydroxylation of the cyclohexane ring was carried out by using the N-phenylcarbamate derivative of cyclohexanol, which conferred the necessary amount of lipophilic character to the substrate. The product was 4-hydroxycyclohexyl N-phenylcarbamate, which was characterized by oxidation to 4-oxocyclohexyl N-phenylcarbamate and comparison with an authentic sample prepared from 4-hydroxycyclohexanone.[9]

Experimental Section[10]

Bioconversion of Cyclododecanol. The bioconversion medium contained 20 g of cornsteep liquor (60% solids) and 10 g of commercial dextrose per liter (tap water) adjusted to pH 4.85. Lard oil was used as an antifoam preventive. In a typical fermentation the sterilized medium (100 l.) was inoculated with a 72-hr vegetative growth of *Sporotrichum sulfurescens*, ATCC 7159, and incubated with thorough agitation at a temperature of about 28°, using a rate of aeration of 5 l./min. After 24 hr of growth, a solution of 20 g of cyclododecanol in 200 ml of N,N-dimethylformamide was added to the inoculated medium. After an additional 72-hr period of incubation, the beer and mycelium were separated by filtration. The mycelium was washed with water, and the wash water was added to the beer filtrate. The filtrate was extracted with four 25-l. portions of methylene chloride. The combined extracts were washed with 25 l. of distilled water, and the solvent was removed by distillation to give a crude residue containing the bioconversion products.

Isolation of Cyclododecanediones. The crude residue was dissolved in 100 ml of acetone and oxidized for 10 min with excess chromic acid (Jones' reagent) (about 30 ml) at 35–40°. The excess chromic acid was destroyed by adding 10 ml of 2-propanol, and the reaction mixture was diluted with 250 ml of water and extracted intensively with methylene chloride. The combined extracts were washed with 150 ml of water and the solvent was removed by distillation under reduced pressure to give about 20 g of a semicrystalline residue containing cyclododecane-1,5-dione, cyclododecane-1,6-dione, and cyclododecane-1,7-dione.

This mixture was dissolved in benzene and chromatographed over Florisil. Elution was with petroleum ether containing increasing amounts (2–10%) of acetone.

Paper chromatographic analysis[11] of eluate fractions showed that 1,5-dione, 1,6-dione, and 1,7-dione were eluted in that order, but that separations were not complete.

The 10% acetone–petroleum ether eluate fractions were combined and recrystallized from acetone–petroleum ether to give 2.32 g of cyclododecane-1,7-dione, mp 132–135°. Further recrystallization of this substance from the same solvent pair afforded an analytical sample of cyclododecane-1,7-dione, mp 134–136°.

Anal. Calcd for $C_{12}H_{20}O_2$: C, 73.43; H, 10.27. Found: C, 73.66; H, 9.99.

The 2% acetone–petroleum ether eluate material was rechromatographed on Florisil, eluting with 1–2% acetone–petroleum ether. An appropriate fraction (1% acetone–petroleum ether eluate) of this chromatogram was twice rechromatographed over alumina (Merck Reagent), eluting with 0.5–1% acetone–petroleum ether to give 110 mg of crude crystals, mp <60°, which was recrystallized from hexane to give 50 mg of cyclododecane-1,5-dione, mp 64–65°.

Anal. Calcd for $C_{12}H_{20}O_2$: C, 73.42; H, 10.27. Found: C, 73.91; H, 10.19.

In another similar experiment in which 25 g of cyclododecanol was biooxygenated, and the extract residue chromatographed on Florisil, elution with 2–5% acetone–petroleum ether, gave material that was crystallized from acetone–petroleum ether to give 2.81 g of cyclododecane-1,6-dione, mp 91–95°. For analysis a sample was recrystallized twice from acetone–petroleum ether to mp 94.5–95.5° (lit.[4] mp 94–95°).

Anal. Calcd for $C_{12}H_{20}O_2$: C, 73.43; H, 10.27. Found: C, 73.64; H, 9.99.

Bioconversion of Cyclotridecanol. Cyclotridecanol (2 g) was subjected to the action of *Sporotrichum sulfurescens* as descibed above. The same type of work-up procedure afforded a mixture of diones that was dissolved in benzene and chromatographed over Florisil. Gradient elution of the column was effected with petroleum ether containing increasing proportions of acetone, ranging from 0 to 6%. The major product fractions were combined and crystallized from ether–hexane to give 95 mg of cyclotridecane-1,7-dione, mp 88–90°.

Anal. Calcd for $C_{13}H_{22}O_2$: C, 74.24; H, 10.54. Found: C, 74.09; H, 10.24.

Bioconversion of Cyclotetradecanol. Cyclotetradecanol (2 g) was subjected to the action of *Sporotrichum sulfurescens* as described above. The same type of work-up procedure afforded a mixture of diones that was chromatographed in benzene over Florisil, eluting (gradient) with 34 250-ml fractions of petroleum ether containing increasing proportions of acetone, ranging from 0 to 6%. The early fractions contained 0.451 g of cyclotetradecanone resulting from unconverted starting material. Later fractions contained

(9) E. R. H. Jones and F. Sondheimer, *J. Chem. Soc.*, 615 (1949).

(10) All melting points were determined using a Fisher-Johns block. "Petroleum ether" refers to a product, bp 60–70°, of the Skelly Corp. called Skellysolve B. Florisil is a synthetic magnesium silicate product of the Floridin Co., Warren, Pa.

(11) L. M. Reineke, *Anal. Chem.*, 28, 1853 (1956). The CFS system was used.

0.22 g of crystalline product that was recrystallized from acetone–hexane to give 120 mg of cyclotetradecane-1,6-dione, mp 92°.

Anal. Calcd for $C_{14}H_{24}O_2$: C, 74.95; H, 10.78. Found: C, 75.20; H, 10.94.

General Procedure for Baeyer–Villiger Degradation and Fragment Identification. 1,*x*-Cycloalkanedione (0.196 g) was added to a solution of 1 ml of peroxytrifluoroacetic acid chilled in an ice–methanol bath. The dione was dissolved by gentle mixing, the flask removed from the cold bath, allowed to warm to room temperature, and again returned to the cold bath. When the solution no longer warmed quickly to room temperature upon removing from the cold bath, it was allowed to stand at room temperature for 30 min and diluted with 20 ml of water. Saturated aqueous potassium iodide (1 ml) was added and the mixture stirred with 25 ml of methylene chloride while adding saturated sodium bisulfite solution until the color of liberated iodine was discharged. The mixture was exhaustively extracted with methylene chloride, and the extract washed with bicarbonate solution and water, dried (sodium sulfate), and the solvent removed to give a colorless oily residue of mixed lactones. Complete conversion to lactone was shown by infrared spectra: no carbonyl absorption at 1680–1725 cm^{-1} but showing strong lactone adsorption at 1745 cm^{-1} and a broad ester band at 1275 cm^{-1}.

Hydrolysis of Lactones and Separation of Fragments into Diols, Dibasic Acids, and Hydroxy Acids. The mixed lactones from the above reaction were heated at reflux for 30 min in a solution of 10 ml of methanol and 2 ml of 2 *N* aqueous sodium hydroxide. The methanol was allowed to evaporate from the solution in a beaker at room temperature and the aqueous residue was diluted to 15 ml with water and placed in an apparatus for continuous ether extraction. The sparingly ether-soluble neutral diol fraction was extracted into ether solution during 1–2 hr. The extract was dried, and the ether was removed at atmospheric pressure to leave a residue of diols. The presence of only OH bands and CH_2 bands in the infrared further substantiated the nature of these fragments which were identified either as chemical entities or by oxidation to dicarboxylic acids, converted to methyl esters, and examined by vpc.[12]

The aqueous phase remaining in the extraction apparatus was acidified with 2 *N* sulfuric acid and continuous ether extraction gave an ether solution of dibasic acid and hydroxy acid fragments. In some instances these were identified as chemical entities. More generally, they were converted to methyl esters by treatment with an ether solution of diazomethane. Chromatography on Florisil readily separated these into dibasic acid methyl ester and hydroxy acid methyl esters. The former were identified by vpc. The hydroxy acid methyl esters were oxidized with Jones' reagent and treated with an ether solution of diazomethane to give the dibasic acid methyl esters which were in turn identified by vpc. That no additional degradation took place during the oxidation of the diols or hydroxy acids was shown by subjecting suberic acid to the oxidation conditions used. No smaller fragments (as dimethyl esters) were found.

1,8-Dioxacyclotetradecane-2,9-dione. Baeyer–Villiger oxidation of 0.588 g of cyclododecane-1,7-dione by the general procedure described above gave 0.640 g of a solid lactone mixture. Recrystallization from ether–hexane gave 0.257 g of 1,8-dioxacyclotetradecane-2,9-dione, mp 111–113°.

Anal. Calcd for $C_{12}H_{20}O_4$: C, 63.13; H, 8.83. Found: C, 63.01, H, 8.87.

Cyclotetradecane-1,7-dione and Cyclotetradecane-1,8-dione from the Hydration of 1,8-Cyclotetradecadiyne. A mixture of 90 ml of aqueous 70% acetone, 0.60 ml of concentrated sulfuric acid, 0.60 g of mercuric sulfate, and 22.56 g of 1,8-cyclotetradecadiyne was stirred and heated at reflux for 18 hr. (In a previous experiment infrared examination of an aliquot residue obtained after 1.5-hr heating showed the ketone was being formed but that the reaction was far from complete.) The mixture was cooled, diluted with 600 ml of water and the crystalline product recovered by filtration, washed with water, and dried. This was combined with a second crop obtained by concentration of the filtrate to give 25.81 g of mixed diones. This mixture of 1,7- and 1,8-diones in benzene was chromatographed over Florisil. Gradient elution with petroleum ether plus increasing proportions of acetone from 0 to 4%, and finally with petroleum ether + 10% acetone gave material from the early fractions that was recrystallized from ether to yield 10.86 g of cyclotetradecane-1,8-dione, mp 149° (lit.[7] mp 149°).

Anal. Calcd for $C_{14}H_{24}O_2$: C, 74.95; H, 10.78. Found: C, 75.20; H, 10.78.

Material eluted in the latter fractions was recrystallized from ether–hexane to give 10.46 g of cyclotetradecane-1,7-dione, mp 103–104°.

Anal. Calcd for $C_{14}H_{24}O_2$: C, 74.95; H, 10.78. Found: C, 74.79; H, 10.62.

4-Oxocyclohexyl N-Phenylcarbamate. A. Cyclohexyl N-phenylcarbamate (2 g) was subjected to the action of *Sporotrichum sulfurescens* as described above. The crude extracted product was chromatographed on Florisil and eluted with 25% acetone in petroleum ether. Recrystallization from acetone–petroleum ether gave 0.73 g of crude **4-hydroxycyclohexyl N-phenylcarbamate**, mp 165–168°.

Oxidation of 0.5 g of the 4-hydroxycyclohexyl N-phenylcarbamate from the fermentation procedure with Jones' reagent gave, after recrystallization from acetone–petroleum ether, 0.38 g of 4-oxocyclohexyl N-phenylcarbamate, mp 135–137°.

Anal. Calcd for $C_{13}H_{15}NO_3$: C, 66.93; H, 6.48; N, 6.01. Found: C, 66.50; H, 6.71; N, 5.84.

B. 4-Hydroxycyclohexanone [1 ml; bp 87–88° (0.3 torr)], prepared by the method of Jones and Sondheimer,[9] was heated on a steam bath with 2 ml of phenyl isocyanate for 15 min, cooled, and washed with petroleum ether. The partly crystalline residue was chromatographed on Florisil, eluted with 10% acetone–petroleum ether, and recrystallized from acetone–petroleum ether to give 0.37 g of 4-oxocyclohexyl N-phenylcarbamate, mp 134–136°, identical in infrared spectrum with the sample derived from the fermentation process.

(12) The chromatograms were carried out using a 2-ft, stainless steel, 0.25-in. column packed with 20% polyester LAC 446 on 60–80 mesh Chromosorb P (w/w). An F & M Model 300 apparatus was used with the temperature programmed at 6.4°/min from 150 to 205° and then held at 205°. The helium flow rate was 35–40 cc/min, with the injection port temperature at 263° and the detector block at 265°. The major components were compared with authentic samples, both separately and by admixture. The column used has been described by C. H. Orr and J. E. Callen, *Ann. N. Y. Acad Sci.*, **72** (13), 649 (1959), and by A. J. Fulco and J. F. Mead, *J. Biol. Chem.*, **234**, 1411 (1959).