the suspension until all of the solid bromine addition compound was converted to the heavy orange oil, tris(*n*-butyl) phosphine sulfide hydrobromide. The oil, following separation from the hexane layer, was taken up in ether, and washed cautiously with aqueous sodium carbonate until carbon dioxide evolution ceased. The ether layer was dried over anhydrous sodium carbonate, and following evaporation of the ether, the residue was carefully vacuum distilled. A number of distillations were necessary to obtain a pure product in yields of 25–40%. Tris(*n*-butyl)phosphine sulfide is a pale yellow oil possessing the following properties: b.p. 137–138° at 1.1 mm.; n_D^{25} 1.4945; d_{24}, 1.0339 g./ml.

Anal. Calcd. for $C_{12}H_{27}PS$: C, 61.49; H, 11.61; P, 13.22; S, 13.68. Found: C, 61.69; H, 11.76; P, 13.28; S, 13.83.

Tris(*n*-butyl)phosphine, tris(*n*-butyl)phosphine oxide, and tris(*n*-butyl)phosphine sulfide are characterized by infrared absorption bands at 718 and 742 cm.$^{-1}$ which is the region of the P—C and the P—S stretch. However, tris(*n*-butyl)-phosphine sulfide shows a much stronger intensity in the 742 cm.$^{-1}$ band than do the other compounds. This suggests that the P—S and P—C vibrations in the tris(*n*-butyl)-phosphine sulfide molecule overlap at this frequency.

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Synthesis of D,L-Caldariomycin[1]

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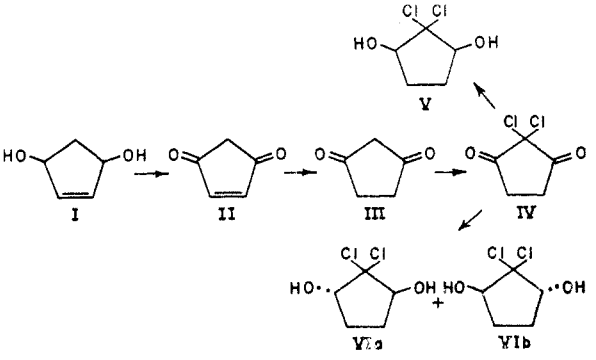
Studies have been initiated in this laboratory into the biosynthesis of caldariomycin,[4a-e] a chlorine-containing metabolite of the mold, *Caldariomyces fumago*. Clutterbuck *et al.*[5] isolated this compound in 1940 and proposed 2,2-dichloro-1,3-cyclopentanediol as the most likely structure for caldariomycin. Caldariomycin is optically active, therefore the natural product was designated as one of the *trans*-hydroxyl isomers (VIa or VIb). Since the structure assignment was based largely on degradative studies and represented a choice between possible alternatives, we proceeded to establish the structure of caldariomycin by synthesis.

We now wish to report the synthesis of a racemic mixture of the *trans*-hydroxyl isomers of caldariomycin by the following steps:



3,5-Cyclopentenediol (I) was separated from a mixture of 3,4- and 3,5-cyclopentenediols[6] by fractional distillation and was oxidized with chromic acid in acetone according to the procedure of DePuy and Zaweski.[7] The product, 3,5-cyclopentenedione (II), obtained in 45% yield, was then reduced with hydrogen over 10% palladium-on-charcoal in chloroform to yield 1,3-cyclopentanedione (III). After recrystallization of III from ethyl acetate, III was chlorinated with a slight excess of sulfuryl chloride. After recrystallization from ether and sublimation, a compound was obtained in 80% yield giving the correct analysis of 2,2-dichloro-1,3-cyclopentanedione (IV). In addition, the strong ultraviolet absorption associated with III had been almost completely eliminated, and a sole carbonyl band was observed in the infrared spectrum at 5.62 μ. Both these spectral features are indicative of the hindrance of any resonance in the formerly highly enolized β-diketone grouping. On the basis of this evidence and the analytical data, the compound was designated as 2,2-dichloro-1,3-cyclopentanedione.

Hantzsch[8] has proposed structure IV for a compound, m.p. 118°, obtained from a base-catalyzed chlorination of phenol. In contrast to IV obtained by the chlorination of III, the Hantzsch product exhibited color with ferric chloride reagent. This latter result would not be expected of IV, since enol formation is strongly inhibited in this compound. In addition, prior to carrying out the present synthesis, we attempted to prepare IV by Hantzsch's procedure without success. It seems most probable, therefore, that Hantzsch incorrectly assigned structure IV to his compound.

When the dichloro diketone (IV) was reduced

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(4) (a) P. D. Shaw and L. P. Hager, *J. Am. Chem. Soc.*, 81, 1011 (1959); (b) P. D. Shaw, J. R. Beckwith, and L. P. Hager, *J. Biol. Chem.*, 234, 2560 (1959); (c) P. D. Shaw and L. P. Hager, *J. Biol. Chem.*, 234, 2565 (1959); (d) P. D. Shaw and L. P. Hager, *J. Am. Chem. Soc.*, 81, 6527 (1959); (e) J. R. Beckwith and L. P. Hager, Abstracts of Papers presented at American Chemical Society Meeting, Atlantic City, N. J., September 1959, p. 17C.

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(6) Obtained from Columbia-Southern Chemical Co., 1 Gateway Ctr., Pittsburgh 22, Pa.

(7) C. H. DePuy and E. F. Zaweski, *J. Am. Chem. Soc.*, 81, 4920 (1959).

(8) A. Hantzsch, *Ber.*, 22, 1238 (1889).

with sodium borohydride, a compound was obtained in 75% yield giving the analysis $C_5H_8O_2Cl_2$. Although this formula is identical with that of caldariomycin, the infrared spectrum of the reduction product was similar but differed significantly from that of the natural product. The OH stretching at 2.78 μ, the CH stretching at 3.41 μ, and the $-CH_2-$ deformation at 6.90 μ were essentially the same in both cases, while the C—O stretching (or OH deformation) appeared at 8.94 μ with caldariomycin and at 9.05 μ with the synthetic compound. In addition, there were noticeable variations in the fingerprint region. The two spectra were close enough to suggest that this compound was, in all likelihood, 2,2-dichloro-*cis*-1,3-cyclopentanediol (V).

A lithium aluminum hydride reduction of the dichloro diketone (IV) in ether also yielded the *cis* compound (V) in lower amounts (50%); but, in addition, there was obtained 20% of a compound giving the identical analysis and the same infrared spectrum as natural caldariomycin. The purified compound melted at 89–90°, whereas caldariomycin melts at 121°. However, this was to be expected as caldariomycin is optically active, and the synthetic compound would be a racemic mixture of the *trans*-hydroxyl isomers (VIa and VIb).

It was found that caldariomycin is far less soluble in chloroform than the synthetic product (VIa and VIb), and this solubility difference was taken advantage of in an isotope dilution experiment designed to prove that the former compound was indeed one half of the racemic pair. This process involved the utilization of biologically prepared Cl^{36}-labeled caldariomycin. Equal amounts of Cl^{36}-labeled caldariomycin and the synthetic racemic mixture were combined and recrystallized several times from chloroform. The resulting specific radioactivity of the crystals upon successive recrystallizations is presented in Fig. 1. As may be observed, the constant specific activity of the caldariomycin after seven recrystallizations is equal to the theoretical amount assuming that the Cl^{36}-labeled caldariomycin had equilibrated with half its weight of unlabeled material. It may be concluded, therefore, that caldariomycin is identical with 50% of the synthetic *trans* isomers obtained by the lithium aluminum hydride reduction of 2,2-dichloro-1,3-cyclopentanedione.

Further effort was made to separate the synthetic D,L-caldariomycin into its two optical antipodes. Reaction of caldariomycin with two equivalents of *d*-camphor-10-sulfonyl chloride yielded a product which gave a correct analysis for the di-*d*-camphor sulfonate derivative of caldariomycin. When the same esterification was performed on the synthetic D,L-caldariomycin, a crystalline product was obtained, having the same optical rotation as the caldariomycin derivative. Upon admixture of the two esters, there was no depression in melting point.

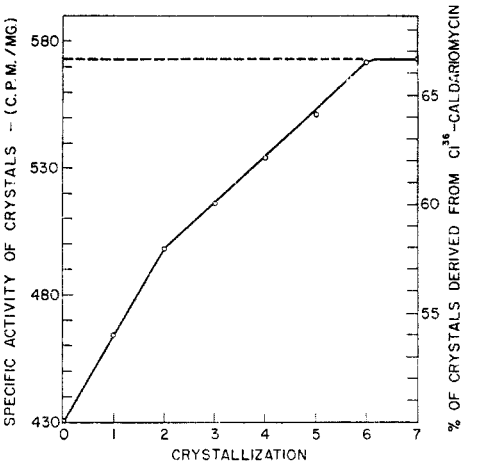

Fig. 1. Recrystallization of Cl^{36} labeled caldariomycin with synthetic D,L-caldariomycin. Forty milligrams of Cl^{36}-labeled natural caldariomycin (860 c.p.m./mg.) was mixed with 40 mg. of synthetic D,L-caldariomycin and repeatedly crystallized from chloroform until caldariomycin, m.p. 121.5°, of constant specific radioactivity was obtained (seven crystallizations). After four crystallizations the melting point was 118–120°. The theoretical specific radioactivity (– – –) of the crystals, assuming a dilution of the Cl^{36}-labeled caldariomycin with one half its weight of unlabeled material, would be 66.6% that of its original specific activity.

Since caldariomycin is quite base labile, the ester derivatives have to be saponified by methods other than alkaline hydrolysis. Limited attempts to cleave the sulfonic acid ester derivatives with lithium aluminum hydride and produce caldariomycin were unsuccessful.

The equivalence of the derivatives of caldariomycin and a portion of the synthetic product plus the radioactive dilution experiment provide conclusive evidence that caldariomycin is identical with one half of the synthetic racemic pair (VIa or VIb). It has thus been established that caldariomycin has the structure 2,2-dichloro-*trans*-1,3-cyclopentanediol as originally postulated by Clutterbuck *et al.*[5]

EXPERIMENTAL[9]

3,5-Cyclopentanedione. 3,5-Cyclopentanediol was oxidized with chromic acid in acetone according to the procedure of DePuy and Zaweski.[7]

1,3-Cyclopentanedione. Palladium-charcoal catalyst (750 mg.) was added to 3,5-cyclopentanedione (7.5 g.) in chloroform (50 ml.), and the mixture was stirred under a hydrogen atmosphere until the theoretical amount of hydrogen uptake had been achieved. It was found that on allowing the mixture to remain longer under hydrogen, the uptake approached twice theoretical and the yield was markedly diminished. The palladium-charcoal catalyst was filtered and repeatedly

(9) Melting points (uncorrected) were observed on a Fisher-Johns melting point apparatus. Infrared spectra were recorded on a Perkin-Elmer Model 21 spectrophotometer. Analyses were performed by Dr. Nagy, Microanalytical Laboratories, Massachusetts Institute of Technology, Cambridge, Mass., and by the Microanalytical Laboratory, University of Illinois, Urbana, Ill.

extracted with hot acetone until no more product was obtained. The combined acetone and chloroform solutions were evaporated to dryness, and after two crystallizations from ethyl acetate, 2.6 g. of 1,3-cyclopentanedione (36%) was obtained, m.p. 151.5–152.5°, $\lambda_{max}^{0.1N\ HCl}$ 242 mμ ε 20,000.

2,2-Dichloro-1,3-cyclopentanedione. To 1,3-cyclopentane dione (1 g., 0.01 mole) in a very small amount of dry benzene, was added slowly with stirring a slight excess of sulfuryl chloride (2.0 ml., 0.025 mole). As the reaction proceeded, more benzene was introduced, and when all the material had gone into solution, the benzene was evaporated and the remaining substance recrystallized twice from ether to give 1.34 g. of 2,2-dichloro-1,3-cyclopentanedione (80%), m.p. 83.5°, $\lambda_{max}^{95\%\ ethanol}$ 277 mμ, ε 160.

Anal. Calcd. for $C_5H_4O_2Cl_2$: C, 35.95; H, 2.41; Cl, 42.42. Found: C, 35.70; H, 2.44; Cl, 42.88.

2,2-Dichloro-cis-1,3-cyclopentanediol. 2,2-Dichloro-1,3-cyclopentanedione (100 mg., 0.6 mmole) was added with stirring to an ice-cooled solution of sodium borohydride (29 mg., 0.76 mmole) in water (5 ml.) and isopropyl alcohol (5 ml.). Water was added after 20 min.; the solution extracted with ether three times and the ether solution dried. The organic solvents were then evaporated, and the residue was recrystallized from chloroform to give a compound (75 mg., 75%), m.p. 135.5°, giving the correct analysis for 2,2-dichloro-1,3-cyclopentanediol.

Anal. Calcd. for $C_5H_8O_2Cl_2$: C, 35.17; H, 4.70; Cl, 41.43. Found: C, 35.58; H, 4.81; Cl, 41.08.

Although the infrared spectrum of this compound, $\lambda_{max}^{CHCl_3}$ 2.76, 3.41, 9.03, 9.90, 10.22, 11.31, 11.66 μ, was similar to caldariomycin, $\lambda_{max}^{CHCl_3}$ 2.74, 3.41, 8.97, 9.72, 10.29, 11.31., 12.00 μ, it was not identical.

2,2-Dichloro-trans-1,3-cyclopentanediol. In a three necked 50-ml. flask equipped with a reflux condenser, mechanical stirrer, and a flow of nitrogen gas was placed lithium aluminum hydride (550 mg., 15 mmoles) in ether (25 ml.). The mixture was brought to a temperature of −2° and maintained there while 2,2-dichloro-1,3-cyclopentanedione (1.5 g., 9 mmoles) in ether was added dropwise over a 0.5-hr. period. After the addition was completed, the reaction mixture was stirred for 10 more min., whereupon the contents of the flask were poured into ice-cold 2*N* hydrochloric acid (100 ml.). The acidic solution was extracted with seven equal volumes of ether. The ether solution was dried over magnesium sulfate and evaporated to dryness. Chloroform (6 ml.) was added to the solid material and the mixture heated until no more of the crystals went into solution. The chloroform solution was held at −20° for several hours for crystallization. The crystals were separated from the supernatant liquor and determined to be identical with the product of the sodium borohydride reduction (750 mg., 50%). When the mother liquor was evaporated down and the remaining crystals sublimed at 95° under reduced pressure (water aspirator), a compound (300 mg., 20%), m.p. 89–90°, was obtained giving the correct analysis for caldariomycin and exhibiting the identical infrared spectrum as the natural product.

Anal. Calcd. for $C_5H_8O_2Cl_2$: C, 35.17; H, 4.70; Cl, 41.43. Found: C, 35.58; H, 4.83; Cl, 41.10.

d-Camphor-10-sulfonyl chloride. *d*-Camphor-10-sulfonic acid (1 g., 0.43 mmole) was treated with thionyl chloride (1 ml., 1.4 mmoles) and heated at 60° until the reaction had stopped. The solution was then poured into water, and the solid material separated by centrifugation. The residue was recrystallized once from acetone-water to give 970 mg. (90%), m.p. 67–70°.

Caldariomycin-bis-d-camphor sulfonate. To a solution of caldariomycin (100 mg., 0.59 mmole) in anhydrous pyridine (0.5 ml.) in a 15-ml. glass centrifuge tube was added *d*-camphor-10-sulfonyl chloride (300 mg., 1.2 mmoles). The tube warmed immediately and a precipitate formed. After 5 min., excess 2*N* hydrochloric acid (5 ml.) was added; and the mixture vigorously stirred. The precipitate was obtained by centrifugation and dried overnight under reduced pressure over phosphorus pentoxide. A sample recrystallized from 95% ethanol melted at 142–143°, $[\alpha]_D^{25}$ +36° (acetone).

Anal. Calcd. for $C_{25}H_{36}O_8S_2Cl_2$: C, 50.08; H, 6.01; Cl, 11.85. Found: C, 50.14; H, 6.28; Cl, 12.08.

Reaction of D,L-*caldariomycin with d-camphor-10-sulfonyl chloride.* The synthetic D,L-caldariomycin (204 mg., 1.2 mmoles) was mixed with the *d*-camphor-10-sulfonyl chloride (620 mg., 2.4 mmoles) according to the procedure used for the natural material. A product was obtained (189 mg.), m.p. 125–135°, which, after 4 recrystallizations from acetone-water, melted at 138–142°, $[\alpha]_D^{25}$ +37°. Upon admixture of this material with the similar derivative of caldariomycin, no depression of the melting point was observed, m.p. 139–143°.

The infrared spectra of the dicamphor sulfonate derivatives of natural caldariomycin and the synthetic product were identical, $\lambda_{max}^{CHCl_3}$ 3.38, 5.73, 7.23, 8.48, 10.35, 11.22, 11.82 μ.

Attempted cleavage of caldariomycin–camphor sulfonate derivative. To a solution of lithium aluminum hydride (74 mg., 2 mmoles) in anhydrous chloroform (20 ml.) was added caldariomycinbis-*d*-camphor sulfonate (200 mg., 0.33 mmole) in anhydrous chloroform (20 ml.). The mixture was stirred for 1 hr. at 20° and then added carefully to water (50 ml.). The water layer was extracted several times with ether; the ether solutions washed once with water, dried over magnesium sulfate, and evaporated to dryness. The residue was redissolved in chloroform (0.5 ml.) and cooled to −20°. No caldariomycin appeared even upon seeding.

Cl^{36}-labeled caldariomycin. *Caldariomyces fumago* was grown on the Czapek-Dox medium described by Clutterbuck *et al.*[5] in the presence of potassium chloride[36] (10,000 c.p.m./μmole). Caldariomycin, labeled with Cl^{36}, was isolated as previously described.[5]

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The Effect of Ring Substituents on Prototropic Isomerism in Phenylhydrazones[1]

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The wide use of arylhydrazones as analytical derivatives of aldehydes and ketones for spectroscopic analysis necessitates an understanding of any changes in structure which may occur in solution. Any isomerization may require the reporting of a time factor for purposes of obtaining reproducible spectra of these derivatives.

Thus phenylhydrazones of aliphatic aldehydes and ketones have been shown[4] to exist originally as the hydrazone isomer (I) and to isomerize rapidly in solution to the more stable benzeneazoalkane (II).

(1) Presented at the American Chemical Society Convention (Organic Division) in St. Louis, Mo., March 27, 1961.

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(3) National Defense Education Act Fellow.

(4) R. O'Connor, *J. Org. Chem.*, 26, 4375 (1961).