methylene chloride containing 2.3 g. (6.0 mmoles) of the above residue V. During the addition the mixture boiled gently and refluxing was continued for an additional hour. After the solution had cooled to room temperature, the liquid layer was removed by careful decantation, and the salts were extracted five times with methylene chloride. The extracts were combined with the decanted solution and washed with saturated sodium chloride solution, filtered and dried over magnesium sulfate. The drying agent was removed by filtration, and the chloroform was removed by evaporation under reduced pressure leaving a semi-solid residue consisting of the acid–ester VI. This residue showed strong infrared absorption in the carbonyl region. The ultraviolet spectrum, however, was transparent between 210 and 400 mμ. Electrometric titration showed the presence of a carboxyl group. Addition of alkali followed by back titration indicated that two equivalents of ester groups had been hydrolyzed.

The ester–acid VI was stirred with 7 ml. of methanol and a solution of 0.55 g. of sodium hydroxide in 30 ml. of water (*p*H 11.8). After 1 hr. additional alkali (5.0 ml. of water containing 370 mg. of sodium hydroxide) was added. After the solution had stood for 4 hr. (*p*H 11.7) most of the methanol was removed by evaporation under reduced pressure, and the *p*H was adjusted to 9.7 by addition of 8.15 ml. of 1.0 *N* hydrochloric acid. The methanol was removed by evaporation, and the residue was extracted continuously overnight with ether.

The ether extract was concentrated and dried over magnesium sulfate. The drying agent was removed by filtration, and the ether was removed from the filtrate by evaporation under reduced pressure. One hundred and fifty milligrams of a crystalline residue of the C_{12}-tetrol VII was obtained, m.p. 146–148°, $[\alpha]^{25}D$ −1.6° (*c* 1, methanol).

Anal. Calcd. for $C_{12}H_{26}O_4$: C, 61.50; H, 11.18; C–CH_3 (4), 25.68; mol. wt., 234. Found: C, 61.64; H, 11.30; C–CH_3, 21.62; mol. wt., 230.7 (ebull. in acetone).

This product gave a negative iodoform reaction and was not oxidized by periodate using the conditions mentioned previously. The infrared spectrum showed strong absorption in the hydroxyl region but none in the carbonyl region.

The aqueous layer was adjusted carefully to *p*H 2.00 using 5.2 ml. of 1.0 *N* hydrochloric acid. The solution was filtered, and the volume of the filtrate was reduced to 30 ml. by evaporation. It was then extracted continuously overnight with ether. The extract was dried twice over magnesium sulfate, filtered and concentrated. The light yellow oily residue crystallized after standing overnight in the refrigerator. The crystals were removed and washed with a little ice-cold ether. The X-ray powder diagram was identical with that of a sample of *meso*-α,α'-dimethyl-β-hydroxyglutaric acid previously isolated from erythromycin.

INDIANAPOLIS, INDIANA

[CONTRIBUTION FROM THE LILLY RESEARCH LABORATORIES]

Erythromycin. XII.[1] The Isolation, Properties and Partial Structure of Erythromycin C

BY PAUL F. WILEY, RICHARD GALE, C. W. PETTINGA AND KOERT GERZON

RECEIVED MAY 29, 1957

The isolation of a third crystalline antibiotic, erythromycin C, from *Streptomyces erythreus* is described. Some of the physical, chemical and biological properties of the antibiotic are discussed and a partial structure proposed.

The isolation of erythromycin and erythromycin B from fermentation broths of *Streptomyces erythreus* has been reported.[2,3] Paper chromatography of some of the fractions obtained in the isolation of erythromycin B indicated the presence of additional antibiotic activity. As a result of this evidence, fermentation broths from certain strains of *Streptomyces erythreus* were investigated in search of a new antibiotic. Examination of these fermentation broths by paper chromatography showed three zones of antibiotic activity (Fig. 1). The least mobile of the zones in a methanol, acetone, water (19:6:75) system is erythromycin B; erythromycin C is the fastest moving component and erythromycin has intermediate mobility. After the presence of erythromycin C in appreciable quantity in a crude fermentation broth of *Streptomyces erythreus* had been shown by paper chromatographic examination, the broth was adjusted to *p*H 9.75 and was extracted with chloroform. The chloroform extract, which contained all three antibiotic substances, was concentrated under reduced pressure to approximately one-tenth of the original volume and allowed to stand overnight in the refrigerator. The crystalline precipitate which formed was found by paper chromatography to contain only erythromycin and erythromycin B activity. The mother liquor was concentrated to dryness under reduced pressure, and the residue was fractionated by Craig countercurrent distribution in order to separate the remaining erythromycin from erythromycin C. A 60-tube, all glass apparatus was used with a solvent system prepared by mixing twenty parts of methyl isobutyl ketone, one part of acetone and twenty parts of 0.1 *N* phosphate buffer at *p*H 6.5. A typical distribution curve is shown in Fig. 2. The fractions shown to contain erythromycin C by paper chromatography were concentrated under reduced pressure until only the aqueous phase remained. This solution was adjusted to *p*H 9.75 and extracted with chloroform. The erythromycin C was crystallized from the concentrated chloroform solution.

It was also possible to separate erythromycin and erythromycin C by chromatography on a cellulose column. A mixture of the two antibiotics in acetone solution was adsorbed on a small amount of cellulose powder and dried. The powder was then packed in a thin band at the top of a previously prepared cellulose column. Development of the column with dilute ammonium hydroxide solution saturated with methyl isobutyl ketone gave at first fractions containing no antibiotic activity followed by fractions containing erythromycin C. The

(1) Previous paper in this series: "Erythromycin. XI. Structure of Erythromycin B," P. F. Wiley, M. V. Sigal, Jr., O. Weaver, R. Monahan and K. Gerzon, THIS JOURNAL, **79**, 6070 (1957).

(2) J. M. McGuire, R. L. Bunch, R. C. Anderson, H. E. Boaz, E. H. Flynn, H. M. Powell and J. W. Smith, *Antibiotics and Chemotherapy*, **2**, 281 (1952).

(3) C. W. Pettinga, W. M. Stark and F. R. Van Abeele, THIS JOURNAL, **76**, 569 (1954).

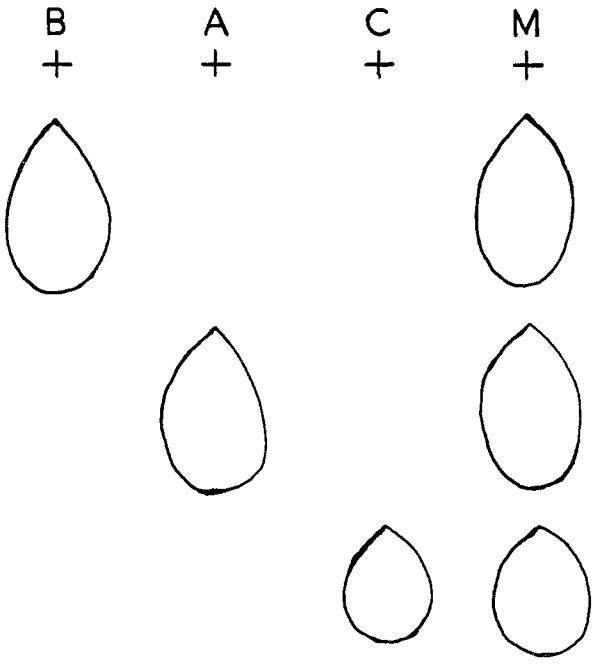

Fig. 1.

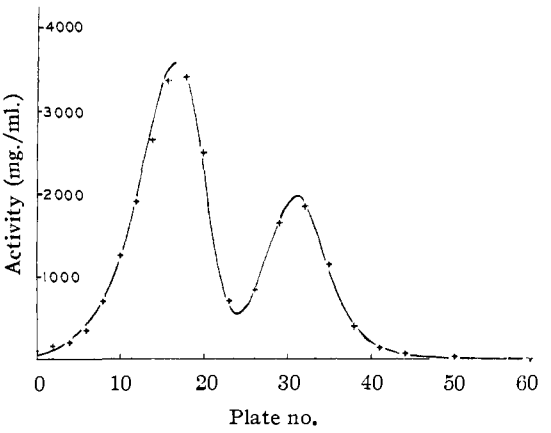

Fig. 2.

erythromycin could be eluted with methyl isobutyl ketone.

Properties of Erythromycin C.—Erythromycin C is similar to erythromycin and erythromycin B in both chemical and physical properties. All are basic compounds relatively insoluble in water and quite soluble in chloroform, acetone and ether. Analysis of erythromycin C gave data consistent with the molecular formula $C_{36}H_{65}NO_{13}$. The spectra are shown on the same plot in Fig. 3 for comparative purposes. The melting point is 121–125° by both Kofler block and capillary tube methods. The microbiological spectrum of erythromycin C is very similar to those of erythromycin and erythromycin B.

Partial Structure of Erythromycin C.—A consideration of the analytical and physical data obtained from erythromycin C indicated that it differs from erythromycin structurally only by the absence of the methoxyl group in the neutral sugar cladinose.[4] Consequently the same degradative procedures used in the erythromycin series[5] were attempted. Acid methanolysis of erythromycin C led to the isolation of erythralosamine (II) and a neutral sugar as is the case with erythromycin.[5] Sodium borohydride reduction to dihydroerythromycin C (III) followed by acid methanolysis formed 5-O-desosaminyldihydroerythronolide (IV) previously isolated from erythromycin.[5] The isolation of these two compounds identical with those derived from erythromycin shows that erythromycin

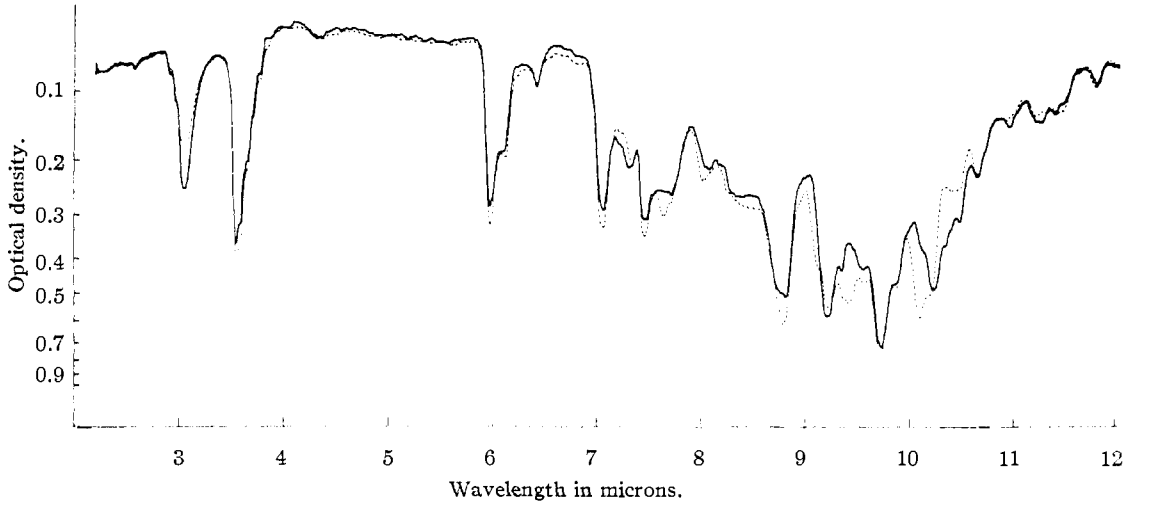

Fig. 3.—Infrared absorption spectra of erythromycin (- - - -), and erythromycin C (——), 5.6% in chloroform, on Perkin-Elmer model 21 spectrophotometer.

methoxy group present in erythromycin is absent. Erythromycin C contains a single basic group with a pK_a' of 8.5 determined by electrometric titration in 66% dimethylformamide and in water. The molecular weight determined by titration is 730 ± 15. The ultraviolet spectrum shows a single broad peak at 292 mμ, ε 108. The infrared spectrum is very similar to that of erythromycin. The two

(4) P. F. Wiley and O. Weaver, THIS JOURNAL, **78**, 808 (1956).

(5) P. F. Wiley, K. Gerzon, E. H. Flynn, M. V. Sigal, Jr., O. Weaver, U. C. Quarck, R. R. Chauvette and R. Monahan, *ibid.*, **79**, 6062 (1957).

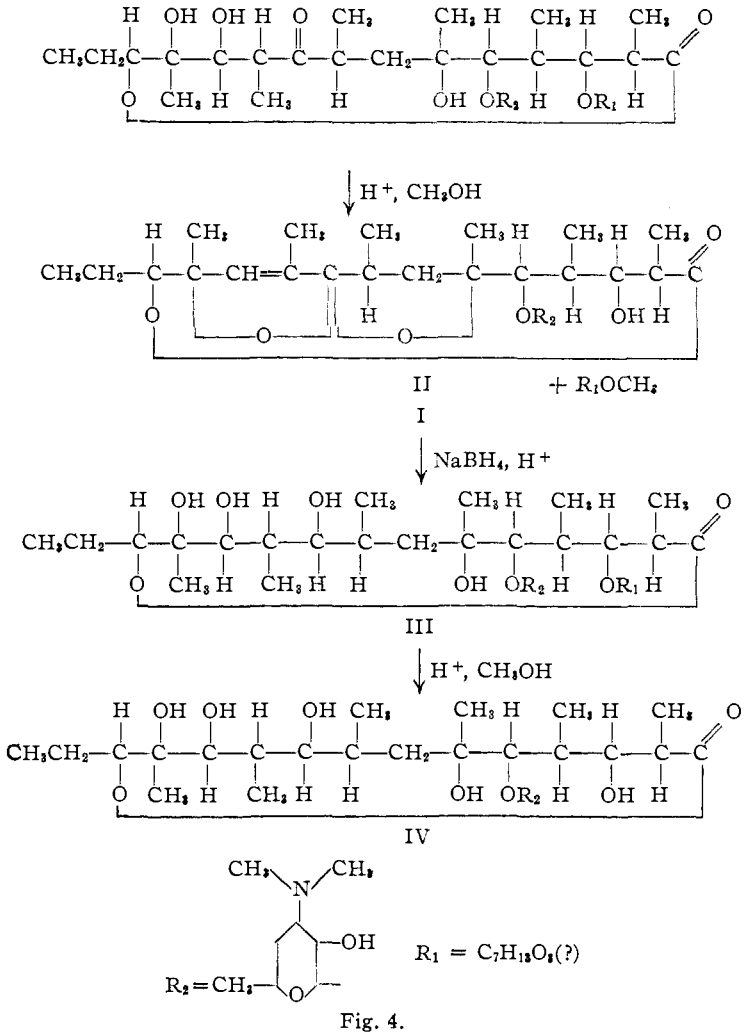

Fig. 4.

and erythromycin C differ only in the neutral sugar moieties and establish the partial structure of erythromycin C to be I.

The lack of a methoxyl group in erythromycin C and the molecular formula indicated that the neutral sugar should have the molecular formula $C_7H_{14}O_4$. This is also the molecular formula of mycarose[6] the neutral sugar existing in magnamycin, and it seemed likely that the two were identical. However, a comparison of the infrared spectra of mycaroside and the methyl glycoside of the erythromycin C sugar showed that they were different compounds. The analytical data obtained for the erythromycin C sugar as its methyl glycoside were not in good agreement with the expected $C_8H_{16}O_4$ formula, but this may be due to a slightly impure sample. Due to the small quantities of material available, purification was inadequate. The analytical data are such that it is believed that the neutral sugar from erythromycin C does have the $C_7H_{14}O_4$ formula.

In the formulas written in the present publication, no implications as to stereochemistry are intended beyond those found in a previous publication.[4]

(6) P. P. Regna, F. A. Hochstein, R. L. Wagner and R. B. Woodward, THIS JOURNAL, **75**, 4625 (1953).

Acknowledgments.—The authors are grateful to Messrs. W. L. Brown, G. M. Maciak, H. L. Hunter and Miss Gloria Beckmann for microanalyses, to Messrs. H. L. Bird and C. T. Pugh for paper chromatographic studies and to Dr. H. Boaz and Messrs. D. O. Woolf and Paul Landis for spectrophotometric data.

Experimental[7]

Extraction.—Eight liters of crude broth containing erythromycin, erythromycin B and erythromycin C was filtered with the aid of 5% Dicolite. The broth filtrate was adjusted to *p*H 9.75 with 40% sodium hydroxide solution and was extracted with four 2-l. portions of chloroform. The chloroform extracts were combined and concentrated *in vacuo* to approximately 100 ml. This concentrate was chilled, and the resulting crystals were removed by filtration. Examination of this crystalline material by paper chromatography showed it to contain 90% erythromycin and 10% erythromycin B. The supernatant contained an activity equivalent of 5,600 mg. of erythromycin.

Countercurrent Distribution.—The supernatant from the above extraction containing an activity equivalent of 5,600 mg. of erythromycin was dried *in vacuo*. The dried residue was then dissolved in 100 ml. of the upper phase of an equilibrated methyl isobutyl ketone, sodium phosphate buffer and acetone system (20:20:1). This was distributed in the above-mentioned solvent system through sixty plates of an all glass Craig countercurrent distribution apparatus having a capacity of 100 ml. per phase in each tube. Upon completion of the distribution an aliquot for assay was drawn from every second tube. The results are plotted in Fig. 2. Paper chromatograms showed that tubes 10–20 contained only erythromycin C, tubes 21–26 showed a mixture of erythromycin and erythromycin C and tubes 27–38 showed only erythromycin. The contents of tubes 10–20 were combined and concentrated *in vacuo* to about 200 ml. and adjusted to *p*H 9.75 with 10% sodium hydroxide solution. The activity was extracted into chloroform and dried over anhydrous sodium sulfate. This was then filtered and concentrated *in vacuo* to about 10 ml. Erythromycin C crystallized from this concentrated solution. The first crop of needle shaped crystals weighed 1 g., m.p. 121–125°.

Anal. Calcd. for $C_{38}H_{65}NO_{13}$: C, 60.05; H, 9.11; N, 1.94; O, 28.88; mol. wt., 720. Found: C, 59.78, 59.72; H, 9.09, 9.11; N, 1.95; O, 29.49 (direct determination); mol. wt., 730 (elect. titr.); CH_3O, none.

Chromatography.—A mixture of erythromycin and erythromycin C was dissolved in acetone and absorbed on 2–3 g. of cellulose powder. This was dried under reduced pressure and placed on a previously prepared column of dry cellulose. The column was developed with 0.01 *N* ammonium hydroxide solution saturated with methyl isobutyl ketone. Fractions (2 ml.) were collected on an automatic fraction collector. Fractions 1–4 contained no antibiotic activity. Fractions 5–15 contained only erythromycin C. The erythromycin could be washed from the column with methyl isobutyl ketone.

Methanolysis of Erythromycin C. (a) **Isolation of Erythralosamine** (II).—Acetyl chloride (2.5 ml.) was added to 100 ml. of absolute methanol. After this solution had cooled, 2 g. of erythromycin C was added, and the solution was allowed to stand at room temperature for 48 hr. It was then poured into a solution of 3 g. of sodium bicarbonate in 100 ml. of water. The solution was made more alkaline by addition of a small amount of sodium bicarbonate. The methanol was removed by evaporation under reduced pressure. The aqueous residue was extracted with four 10-ml. portions of chloroform. After evaporation of the com-

(7) Melting points are uncorrected.

bined chloroform extracts to dryness under reduced pressure, the residue was crystallized from petroleum ether (60–70°). A yield of 0.81 g. (54%) of material melting at 198–200° was obtained. Recrystallization from benzene–petroleum ether (60–90°) raised the melting point to 201–202° identical with that of erythralosamine in the same apparatus. A mixture melting point gave no depression, and the X-ray diffraction patterns were identical.

(b) **Isolation of Neutral Glycoside.**—The aqueous solution from the above experiment was evaporated to dryness under reduced pressure at room temperature. The residue was extracted with four 15-ml. portions of boiling benzene filtering each extract. Evaporation of the combined extracts under reduced pressure left a residue of 0.14 g. This was distilled at a bath temperature of 90–120° under a pressure of 0.05 mm. The infrared spectrum had strong absorption at 2.87 and 2.95 μ and weak absorption in the carbonyl region at 5.9 μ. This spectrum differed from that of methyl mycaroside.

Anal. Calcd. for $C_8H_{16}O_6$: C, 54.54; H, 9.12; CH_3O (1), 17.6. Found: C, 52.54; H, 9.07; CH_3O, 13.6.

Reduction and Methanolysis of Erythromycin C.—A solution of 1 g. of erythromycin C in 6 ml. of methanol was reduced with sodium borohydride and subsequently subjected to acid methanolysis as described for the preparation of X-O-desosaminyldihydroerythronolide.[8] The reduction product (215 mg.) obtained melted at 205–207°. The infrared spectrum and the X-ray diffraction pattern were indistinguishable from those of 5-O-desosaminyldihydroerythronolide[4] prepared from erythromycin.

(8) M. V. Sigal, Jr., P. F. Wiley, K. Gerzon, E. H. Flynn, U. C. Quarck and O. Weaver, THIS JOURNAL, **78**, 388 (1956).

INDIANAPOLIS, INDIANA

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA AT LOS ANGELES]

Polynitrogen Systems from the Hydrazinocarbonic Acids. V.[1] Aminolytic Reactions of N,N-Diphenylcarbamyl Azide[2]

BY F. L. SCOTT[3a] AND M. T. SCOTT[3b]

RECEIVED NOVEMBER 16, 1956

The possible competition between Curtius rearrangement of diphenylcarbamyl azide (Ic) and displacement of its azide function by extraneous bases has been examined. In pyridine or ethanol as solvent the Curtius reaction was dominant, the sole products of aminolysis being 1,1-diphenyl-4-substituted semicarbazides (IV). When various amines were employed as both solvents and reactants, effective competition with the facile intramolecular rearrangement was realized. With amines of b.p. <110° and pK_a's of from 9.7 to 11.2, displacement of the azide moiety of Ic was the sole process encountered yielding ureas of type V. Those bases with b.p.'s from 110–185° and pK_a's from 4 to 9 either resulted in a mixture of azide displacement and Curtius reaction or else in the latter alone. Those bases with b.p.'s >185° (and pK_a's of *ca.* 9–10) formed symmetrically substituted ureas such as VI. The trends operative within the various classes of reaction with pK_a and temperature variations have been explored.

In another paper[1] of this series we formulated the Curtius rearrangement of certain carbamyl azides, *e.g.*, Ia, as involving cyclic transition states of type II (*cf.* ref. 2). We then related the inability to rearrange of some of the substituted carbamyl azides, *e.g.*, Ib, to the incapability of the specific amino groups therein for adequate sustenance of the electronic requirements, *viz.*, 'onium character, that such rearrangement would necessitate. With the very low driving force toward Curtius change that an azide such as Ib then possesses, alternative reactions compete effectually with its possible rearrangement. Such competing reactions may include simple azide displacements[4] (mode B, Fig. 1) or tetrazole closures[5] or perhaps homolytic scissions[6] of the azide function. In general such processes are potentially competitive even with the facile Curtius rearrangements of azides such as Ia, but under the conditions so far investigated the driving forces of this latter class of carbamyl azide toward rearrangement have been sufficiently large so as to inhibit the intrusion of any other mode of reaction upon the Curtius transformation.

The present work was an endeavor to intercept this facile rearrangement. An azide was accordingly chosen, namely, N,N-diphenylcarbamyl azide (Ic) which had been previously[7] observed to undergo the Curtius reaction readily and the extent to which the presence of extraneous bases interfered with the Curtius process was examined. In ethanolic solution, the reactions of Ic with various amines resulted in the exclusive formation of the corresponding 1,1-diphenyl-4-substituted semicarbazides (IV) and thus corresponded to dominance of the Curtius mode of reaction. The same result was observed when pyridine was used as the reaction medium, only therein, under our conditions, the velocity of rearrangement of Ic was roughly fifty times that of the corresponding reaction in refluxing ethanol.[8] When amines of very low nucleophilicity were employed, or when no additional bases were present, both solvents themselves induced Curtius transformations in Ic. While in ethanol the product obtained was 1,1-diphenyl-2-carbethoxyhydrazine[7a]; in pyridine both 1,1,5,5-tetraphenylcarbohydrazide (IVa) and another disproportionation product of formula $C_{19}H_{24}N_3O$ were isolated.

(1) Part IV, this series, F. L. Scott, A. J. Kocjarski and J. Reilly, *J. Org. Chem.*, in preparation.

(2) A previous preliminary communication mentioning some of these results was F. L. Scott, *Chem. & Ind.*, 959 (1954).

(3) (a) To whom inquiries concerning reprints are to be sent, Pennsalt Chem. Corp., P.O. Box 4388, Phila. 18, Pa.; (b) Department of Chemistry, Marymount College, Los Angeles 24, California.

(4) Compare M. S. Newman, S. H. Lee, Jr., and A. B. Garrett, THIS JOURNAL, **69**, 113 (1947).

(5) Compare F. L. Scott, F. C. Britten and J. Reilly, *J. Org. Chem.*, **21**, 1519 (1956).

(6) A. Bertho, *J. prakt. Chem.*, **120**, 94 (1929).

(7) (a) R. Stolle, *Ber.*, **57**, 1065 (1924); (b) R. Stolle, N. Nieland and M. Merkle, *J. prakt. Chem.*, **116**, 192 (1927); (c) R. Stolle, N. Nieland and M. Merkle, *ibid.*, **117**, 185 (1927); (d) R. Stolle and M. Merkle, *ibid.*, **119**, 275 (1928).

(8) This difference is largely due to the temperature differential between the two reactions.