Schroeder.[2a] In the present paper we studied the influence of the quantity of the catalyst on the steric composition of the recovered pigment (Table II).

(i) **Influence of Carbon Dioxide on Some Calcium Hydroxide Chromatograms of Carotenoids.**—If a stream of carbon dioxide is passed through a petroleum ether solution of γ-carotene for five to thirty minutes, no noticeable stereoisomerization takes place. When the solution is poured on a column and developed with the solvent containing 2% acetone, a single zone moves downward with unusual speed at first, due to the formation of carbonate in the top section. Later, when this movement slows down because of the local absence of carbonate, a separation into two very well differentiated zones occurs. The lower zone is unchanged γ-carotene and the upper one is a "complex" which contains 15 to 30% of the initial pigment. The two adsorbates have very similar colors. The upper zone does not migrate further, even if the column is washed with pure acetone; furthermore, it cannot be eluted with alcohol, acetone, dioxane, pyridine, etc. We were able to elute only very small fractions which showed the spectral bands of γ-carotene. In contrast, the unchanged γ-carotene zone can be eluted easily, and gives rise to the formation of a new portion of the complex upon a repeated treatment with carbon dioxide.

These phenomena are not observed if before chromatography the carbon dioxide is removed from the γ-carotene solution by means of a nitrogen stream or evaporation *in vacuo*. They can be reproduced by keeping the pigment solution in a carbon dioxide atmosphere, originating either from a Kipp generator or from Dry Ice.

So far we have observed the appearance of the non-elutable pigment on calcium hydroxide columns only (or on a mixture of the hydroxide and calcium carbonate) but not on pure calcium carbonate, aluminum oxide, zinc carbonate, magnesium oxide, magnesium hydroxide or barium hydroxide. Among the carotenoids tested, lycopene, which requires some reinvestigation, behaves like γ-carotene, as does also the bacterial pigment spirilloxanthin.[6] β-Carotene showed a much smaller effect.

If calcium hydroxide is to be used in quantitative experiments, the solutions should not be kept under carbon dioxide but, preferably, under nitrogen in order to exclude autoxidation.

Summary

The *cis–trans* isomerization of γ-carotene, $C_{44}H_{56}$, (from *Mimulus* and *Gazania* flowers, lower melting form) has been studied by several methods. Some stereoisomers have been tentatively assigned configurations.

PASADENA, CALIFORNIA RECEIVED SEPTEMBER 18, 1944

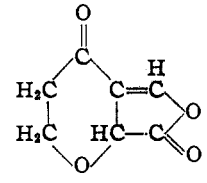
[CONTRIBUTION FROM THE DEPARTMENT OF MICROBIOLOGY, NEW JERSEY AGRICULTURAL EXPERIMENT STATION]

The Mechanism of the Antibiotic Action of Clavacin and Penicillic Acid[1,2]

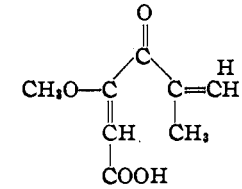
BY WALTON B. GEIGER AND JEAN E. CONN[2a]

Despite the rapidly accumulating information concerning the production of antibiotic substances by microörganisms, their isolation, and their utilization for combating disease, comparatively little still is known of the mode of action of these substances upon bacteria. Among the most important characteristics of these substances is their selective action upon bacteria: some act largely upon Gram-positive forms and to only a very limited extent upon Gram-negative types, whereas others affect alike bacteria within both these groups. Among the substances that belong to the second category, clavacin[3,4,5,6] and penicillic acid[7] occupy a prominent place. Each of these substances is produced by several fungi. Both are active on bacteria belonging to the Gram-positive and Gram-negative types. Both clavacin (I) and penicillic acid (II) are α,β-unsaturated ketones.

Because of the comparatively simple structure of these two compounds, their peculiar antibacterial properties have aroused considerable attention. Of penicillic acid, Oxford[7] wrote," It is not too much to say that there is no feature, or combination of features, in this structure which, on the basis of existing knowledge, would lead one to anticipate an activity of the order found."



I



II

In the course of chemical studies on the structure of clavacin,[8] our attention was directed to a structural feature, common to both penicillic acid and clavacin, that seemed likely to be responsible for their antibacterial activity, namely, the —CH=C—C=O group. Moreover, this part of the molecule is the only structural detail common to both substances. The observation that drew attention to this grouping was the fact that clavacin is inactivated by sulfhydryl compounds such as cysteine or thioglycolate.

The ability of many α,β-unsaturated ketones to react with sulfhydryl compounds was discovered by Posner[9] and may be presented as follows

(1) Journal Series Paper, New Jersey Agricultural Experiment Station, Rutgers University, Department of Microbiology.

(2) These investigations were supported by a grant supplied by The Commonwealth Fund of New York.

(2a) Present address, Department of Bacteriology, University of Iowa, Ames, Iowa.

(3) Waksman, Horning and Spencer, *J. Bact.*, **45**, 233 (1942).

(4) Raistrick, Birkinshaw, Michael, Bracken, Gye and Hopkins, *Lancet*, **245**, 625 (1943).

(5) Hooper, Anderson, Skell and Carter, *Science*, **99**, 16 (1944).

(6) Katzman, Hays, Cain, Van Wyk, Reithel, Thayer, Doisy, Gaby, Carroll, Muir, Jones and Wade, *J. Biol. Chem.*, **154**, 475 (1944).

(7) Oxford, *Chem. & Ind.*, **48** (1942).

(8) Conn and Geiger, *J. Bact.*, **47**, 422 (1944).

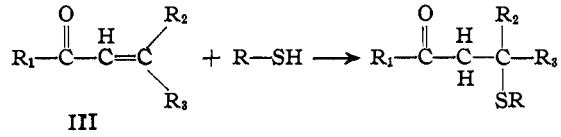
(9) Posner, *Ber.*, **35**, 799 (1902); **37**, 502 (1904).

TABLE I

EFFECT OF SULFHYDRYL COMPOUNDS ON THE BACTERIOSTATIC ACTION OF CLAVACIN AND PENICILLIC ACID

Antibiotic	Sulfhydryl compound	Dilution units[a] per gram of antibiotic agent: *Escherichia coli*	*Staphylococcus aureus*	*Bacillus mycoides*	*Bacillus subtilis*	*Sarcina lutea*
Clavacin	None	300,000	200,000	200,000	200,000	>300,000
	Cysteine	<10,000	....	5,000	60,000	90,000
	Thioglycolate	<3,000	5,000	2,000	5,000	30,000
	Thiosulfate	<2,000	<2,000	2,000	<2,000	....
Penicillic acid	None	20,000	>30,000	30,000	30,000	>30,000
	Cysteine	800	8,000	2,000	5,000	20,000
	Thioglycolate	2,000	5,000	3,000	2,000	10,000
	Thiosulfate	15,000	>30,000	30,000	30,000	>30,000

[a] A dilution unit is that amount of material that will just inhibit the growth of the test organism in 1 ml. of medium.[15]



III

That a substance capable of reacting with sulfhydryl groups should produce antibacterial effects is not surprising, since recent work has amply demonstrated the widespread significance of sulfhydryl compounds in living systems.[10]

Sulfhydryl groups are known to be of exceptional importance in enzyme systems, either as constituents of the actual enzyme protein, or in activators such as glutathione. The fact that the antibacterial activities of clavacin and of penicillic acid depend on their reaction with sulfhydryl groups should be established experimentally in three ways:

1. Penicillic acid and clavacin, when in excess, should eliminate the nitroprusside test given by the free sulfhydryl groups of typical thiol-compounds.
2. Penicillic acid and clavacin should be inactivated by sulfhydryl compounds when the latter are present in excess.
3. Properly constituted synthetic unsaturated ketones should show antibacterial activity comparable to that of the natural antibiotic agents.

The phrase "properly constituted" may be partly defined on the basis of chemical studies of the addition reactions of unsaturated ketones by different investigators.[9,11,12,13,14]

From this work one may conclude that a ketone (III) will undergo addition reactions most readily when R_1 is an aromatic radical such as phenyl, and when R_2 or R_3 is a hydrogen atom.

Experimental

Biological Methods.—The bacteriostatic and fungistatic activities of the unsaturated ketones were determined by the agar-plate streak method,[15] after the compound was dissolved in water or alcohol. When alcohol was used as a solvent, dilutions were chosen in such a manner that no more than 0.3 ml. of alcohol was added to any plate containing 10 ml. of agar.

The effect of sulfhydryl compounds on the bacteriostatic action of unsaturated ketones was determined as follows: The required quantity (usually two molecular proportions) of the sulfhydryl compound was brought to *p*H 7.0 (unless otherwise stated), by neutralization and the addition of a buffer, then diluted with water or alcohol, depending on the solubility of the ketone, and the ketone added, in such quantity as to yield a concentration of 10 mg. per ml. The mixture was kept at 37.5° for twenty-four hours, and tested for bacteriostatic activity, by the agar plate streak method.[15]

For example (Table I, second item), 104 mg. of cysteine hydrochloride was dissolved in 2.5 ml. of a 0.2 *M* phosphate–citrate buffer at *p*H 7.0, and the *p*H readjusted to 7.0 by adding 0.1 *N* sodium hydroxide. A solution of 50 mg. of clavacin in one ml. of water was then added, and the mixture was diluted to 5 ml., incubated, and tested.

Chemical Methods.—To detect chemical reactions between the unsaturated ketone and the sulfhydryl compound, the two substances were mixed as before, except that a 100% excess of the ketone was used. After two and after twenty-four hours, a test for free sulfhydryl groups was made by adding freshly-prepared dilute sodium nitroprusside solution and 3 *N* ammonia. The mesityl oxide, phorone, benzalacetone, benzalacetophenone, furfuralacetone and furfuralacetophenone were Eastman Kodak Co. products. The isophorone was obtained from the Carbon and Carbide Chemicals Corporation, and the indalone from the U. S. Industrial Alcohol Co. Acrylophenone was prepared from β-chloropropiophenone.[16] The clavacin and penicillic acid[17] were the crystalline natural products, the first obtained from *Aspergillus clavatus* and the second from *Penicillium puberulum*.

Results

Clavacin and Penicillic Acid.—Both clavacin and penicillic acid were found to be inactivated by sulfhydryl compounds, as the data in Table I show. Thioglycolate and thiosulfate inactivated clavacin to an extent of 97% or more, and were considerably more effective than cysteine. No large excess of thioglycolate was needed for this inactivation: a 10% excess was quite sufficient. With penicillic acid, both cysteine and thioglycolate were powerful inactivators, but thiosulfate was completely ineffective. Although thiosulfate is an inorganic salt without a true sulfhydryl group, this substance frequently reacts

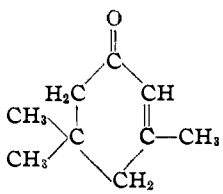
(10) Bersin, *Ergeb. Enzymforsch.*, **4**, 68 (1935).
(11) Ruhemann, *J. Chem. Soc.*, **87**, 17 (1905).
(12) Kohler, *Am. Chem. J.*, **38**, 511 (1907).
(13) Nicolet, THIS JOURNAL, **57**, 1098 (1935).
(14) Chelintsev and Till, *C. A.*, **35**, 6953[2] (1941).
(15) Waksman and Woodruff, *J. Bact.*, **40**, 581 (1940).
(16) Allan, Bell, Bell and Van Allan, THIS JOURNAL, **62**, 656 (1940).
(17) The authors are indebted to Merck and Co. for supplying the crystalline penicillic acid

with organic compounds as if a sulfhydryl group were present.[18]

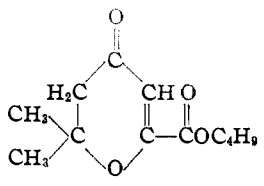
Especially noteworthy is the observation that activity against the Gram-negative *Escherichia coli* was generally more easily and completely abolished than that against the other four organisms, which are Gram-positive. The activity of clavacin against certain other Gram-negative bacteria, namely, *Proteus vulgaris*, *Aerobacter aerogenes*, *Serratia marcescens* and two strains of *Pseudomonas aeruginosa* was also abolished by excess thioglycolate.

Clavacin and penicillic acid, when present in excess, reacted with the sulfhydryl group of cysteine or thioglycolate so rapidly and completely that the nitroprusside reaction was negative in two hours or less at 37.5° and *p*H 4.5. The reaction seemed to be even more rapid at higher *p*H values.

Synthetic Unsaturated Ketones.—The unsaturated ketones examined represent a variety of structural types. Mesityl oxide and phorone are purely aliphatic; isophorone (IV) and indalone (V) are alicyclic; benzalacetone and



IV



V

furfuralacetone have aromatic groups adjacent to the olefinic bond; and acrylophenone, benzalacetophenone, and furfuralacetophenone have aromatic groups adjacent to the carbonyl group.

The data of Table II show that only the last three of these compounds have significant bacteriostatic action. These three compounds, on the basis of the criteria discussed in the introduction, are the ones which would be expected to react most readily with sulfhydryl groups. The most active of the three compounds, acrylophenone, fills these conditions perfectly, since here R_1 is a phenyl group and both R_2 and R_3 are hydrogen atoms. Acrylophenone has as strong a bacteriostatic action against the Gram-positive organisms as clavacin, and is considerably more powerful than penicillic acid. Against the Gram-negative *E. coli*, its activity is less marked, but still considerable, and greater than that of penicillic acid. None of the other synthetic unsaturated ketones, not even the otherwise active benzalacetophenone and furfuralacetophenone, were appreciably bacteriostatic to *E. coli*.

TABLE II

BACTERIOSTATIC ACTION OF α,β-UNSATURATED KETONES

	Dilution units per gram of substance				
Substance	*E. coli*	*S. aureus*	*B. mycoides*	*B. subtilis*	*S. lutea*
Mesityl oxide	<2,000	<2,000	<2,000	<2,000	6,000
Phorone	<3,000	2,000	<3,000	<3,000	3,000
Isophorone	<2,000	<2,000	<2,000	<2,000	<2,000
Indalone	<300	3,000	3,000	5,000	10,000
Benzalacetone	<600	600	600	600	6,000
Furfural-acetone	<3,000	<3,000	<3,000	<3,000	3,000
Acrylophenone	30,000	200,000	300,000	200,000	300,000
Benzalaceto-phenone	<3,000	30,000	50,000	30,000	>100,000
Furfuralaceto-phenone	<3,000	30,000	30,000	30,000	30,000

In Table II are also included data on the antibacterial effect of sodium iodoacetate, a substance commonly used to block sulfhydryl-activated enzyme systems. Its bacteriostatic spectrum is much like that of clavacin, although it is generally less effective.

A more complete survey of the effect of the two antibiotic agents and certain of the unsaturated ketones on Gram-negative forms is given in Table III. Here the resemblance between clavacin and acrylophenone in bacteriostatic properties is again evident. In general, acrylophenone is about one-third or one-tenth as active against the Gram-negative organisms as clavacin. The other synthetic unsaturated ketones are decidedly inferior to acrylophenone, as is also penicillic acid.

Clavacin has been reported by several authors[3,6] to have a fungistatic action. The fungistatic properties of the two antibiotics were compared with those of some unsaturated ketones, with the results given in Table IV. Here also acrylophenone shows activity of the same order of magnitude as clavacin, and benzalacetophenone and particularly furfuralacetophenone also show strong fungistatic action. Penicillic acid has slight fungistatic action.

The proposition that clavacin and penicillic acid owe their activity to reactivity with sulfhydryl groups should, if valid, be extensible to synthetic unsaturated ketones. That is, like these two antibiotics, the synthetic unsaturated ketones—or at least the most strongly bacteriostatic ones—should be inactivated by an excess of a sulfhydryl compound. This prediction is borne out by the experimental data given in Table V. Acrylophenone, which imitates clavacin more closely than does any other synthetic ketone, is inactivated by both cysteine and thioglycolate, but not by thiosulfate. A further similarity to clavacin is that its action against the Gram-negative *E. coli* is more completely inhibited than its activity against the Gram-positive bacteria. The sulfhydryl compounds did not appreciably affect, however, the bacteriostatic properties of benzalacetophenone and furfuralacetophenone. This apparent deviation from theory finds explanation in certain purely chemical experiments.

In Table VI is reported the ability of the two antibiotics and the various unsaturated ketones, when present in excess, to abolish the test for sulfhydryl groups given by cysteine or thioglycolate. From these data it is apparent that with only three of the substances, clavacin, penicillic

(18) Bunte, *Ber.*, **7**, 646 (1874).

TABLE III

BACTERIOSTATIC ACTION OF CLAVACIN, PENICILLIC ACID, AND SYNTHETIC UNSATURATED KETONES UPON GRAM-NEGATIVE BACTERIA

Substance	Dilution units per gram of substance					
	E. coli	*Pseudomonas aeruginosa* I	*Pseudomonas aeruginosa* III	*Pseudomonas fluorescens*	*Proteus vulgaris*	*Serratia marcescens*
Clavacin	300,000	10,000	10,000	10,000	>100,000	30,000
Penicillic acid	20,000	3,000	2,000	3,000	> 30,000	3,000
Acrylophenone	30,000	3,000	2,000	3,000	>100,000	10,000
Benzalacetone	<3,000	1,000	1,000	1,500	1,500	10,000
Benzalacetophenone	<3,000	500	500	500	1,500	500
Furfuralacetophenone	<3,000	<600	<600	<600	10,000	<600

TABLE IV

FUNGISTATIC ACTION OF α,β-UNSATURATED KETONES

Substance	Dilution units per gram of substance					
	Aspergillus niger	*Aspergillus oryzae*	*Rhizopus* sp.	*Trichoderma* sp.	*Fusarium culmorum*	*Ceratostomell ulmi*
Mesityl oxide	<2,000	<2,000	<2,000	<2,000	<2,000	6,000
Phorone	<2,000	<2,000	<2,000	<2,000	<2,000	<2,000
Isophorone	<2,000	<2,000	<2,000	<2,000	<2,000	<2,000
Indalone	<2,000	<2,000	<2,000	2,000	2,000	2,000
Acrylophenone	10,000	10,000	50,000	100,000	100,000	>100,000
Benzalacetone	10,000	15,000	15,000	40,000	1,500	>50,000
Furfuralacetone	6,000	4,000	2,000	4,000	6,000	20,000
Benzalacetophenone	1,500	1,500	50,000	40,000	40,000	>50,000
Furfuralacetophenone	40,000	40,000	40,000	40,000	40,000	>200,000
Clavacin	3,000	3,000	80,000	30,000	3,000	>100,000
Penicillic acid	<1,000	<1,000	<1,000	<1,000	<1,000	3,000

TABLE V

EFFECT OF SULFHYDRYL COMPOUNDS ON THE BACTERIOSTATIC ACTIVITY OF UNSATURATED KETONES

Substance	Sulfhydryl compound	Dilution units per gram of substance				
		E. coli	*S. aureus*	*B. mycoides*	*B. subtilis*	*S. lutea*
Acrylophenone	None	30,000	200,000	300,000	200,000	300,000
	Cysteine	<12,000	12,000	40,000	20,000	120,000
	Thioglycolate	<3,000	8,000	20,000	10,000	30,000
	Thiosulfate	20,000	100,000	100,000	100,000	100,000
Benzalacetophenone	None	<3,000	30,000	50,000	30,000	>100,000
	Cysteine	....	30,000	30,000	30,000	>30,000
	Thioglycolate	....	20,000	20,000	20,000	>30,000
	Thiosulfate	....	70,000	50,000	50,000	>100,000
Furfuralacetophenone	None	<3,000	30,000	30,000	30,000	30,000
	Cysteine	....	20,000	15,000	30,000	>30,000
	Thioglycolate	....	10,000	30,000	10,000	30,000
	Thiosulfate	....	20,000	20,000	30,000	100,000

acid, and acrylophenone, does the reaction with the sulfhydryl compounds go to completion. With the others, the reaction is either incomplete or reversible.

A more quantitative study in which the course of the reaction of the unsaturated ketone with thioglycolic acid was followed by titrating the latter with iodine led to similar conclusions. For example, clavacin and acrylophenone had reacted completely in two hours at 35°, whereas the reaction of benzalacetophenone was only 90% complete after eighteen hours, and benzalacetone showed no signs of reacting. With clavacin, there was evidence for a slow secondary reaction with a second molecule of thioglycolic acid.

Discussion

An interesting parallel between the mechanism of bacteriostasis by clavacin or penicillic acid and that due to mercury compounds affords support for the present hypothesis. Fildes[19] has demonstrated that the antibacterial effects of mercuric chloride were eliminated by an excess of the sulfhydryl compound, glutathione. This was also found to be true of organic mercurials by Nungester, Hood and Warren.[20] Exactly what physiological process is disrupted when clavacin or a mercurial reacts with sulfhydryl groups is not immediately apparent, and possibly it varies with the substance and the organism. It is generally recognized[21,22,23] that destruction of an essential metabo-

(19) Fildes, *Brit. J. Exptl. Path.*, **21**, 67 (1940).

(20) Nungester, Hood and Warren, *Proc. Soc. Exptl. Biol. Med.*, **52**, 287 (1943).

(21) Woods, *Biochem. J.*, **36**, 3 (1942).

(22) Waksman, *Am. J. Public Health*, **34**, 358 (1944).

(23) McIlwain, *Nature*, **153**, 300 (1944).

TABLE VI

NITROPRUSSIDE TEST FOR SULFHYDRYL GROUP OF CYSTEINE OR THIOGLYCOLATE IN THE PRESENCE OF A 100% EXCESS OF AN UNSATURATED KETONE

++++ Strong red color; +++ Moderate red color; ++ Weak red color; + Faint red color; ± Dubious pinkish color; − No color produced; ? Reaction abnormal. Blue color produced.

Ketone	Sulfhydryl compound	Nitroprusside test 2 hours	24 hours
Mesityl oxide	Cysteine	?	?
	Thioglycolate	++++	++++
Phorone	Cysteine	?	?
	Thioglycolate	++++	++++
Isophorone	Cysteine	++++	++++
	Thioglycolate	++++	++++
Indalone	Cysteine	+++	+++
	Thioglycolate	++++	++++
Benzalacetone	Cysteine	++	+
	Thioglycolate	++	+++
Furfuralacetone	Cysteine	++	±
	Thioglycolate	++++	++++
Acrylophenone	Cysteine	+	−
	Thioglycolate	−	−
Benzalacetophenone	Cysteine	++	+
	Thioglycolate	+++	+++
Furfuralacetophenone	Cysteine	+++	±
	Thioglycolate	++++	++++
Clavacin	Cysteine	−	−
	Thioglycolate	+	−
Penicillic acid	Cysteine	−	−
	Thioglycolate	±	±

lite or interference with an enzyme system could account for the activity of antibacterial substances. The definite proof by Hellerman, Chinard and Deitz[24] that the inhibition of the enzyme urease by an organic mercurial is a result of the combination of the latter with sulfhydryl groups of the enzyme gives strong support to these hypotheses.

Acknowledgment.—The authors are indebted to Professor Selman A. Waksman for helpful advice, criticism, and encouragement, and to Miss Dorcas Fasan for a portion of the bacteriological testing.

Summary

1. The antibiotic activities of clavacin and of penicillic acid probably are due to their reaction with the sulfhydryl groups of bacterial enzyme systems or with sulfhydryl-containing metabolites essential to the bacteria.

2. Clavacin and penicillic acid are inactivated by an excess of a sulfhydryl compound.

3. Clavacin and penicillic acid, when present in excess, abolish the nitroprusside reaction of cysteine or thioglycolic acid.

4. Certain synthetic α,β-unsaturated ketones, particularly acrylophenone, closely resemble clavacin, both in their bacteriostatic and fungistatic properties, and in their reactivity toward sulfhydryl compounds.

(24) Hellerman, Chinard and Deitz, *J. Biol. Chem.*, **147**, 443 (1943).

NEW BRUNSWICK, N. J. RECEIVED SEPTEMBER 28, 1944

[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF TEMPLE UNIVERSITY]

The Nitration of Certain Halobiphenyls. IV. Nitro Derivatives of 3-Bromobiphenyl

BY FRANCIS H. CASE

The nitration of 3-chlorobiphenyl according to Mascarelli and Gatti[1] yields a dinitro derivative (m. p. 202–203°) of unknown constitution. We have now shown it to be 3-chloro-4,4′-dinitrobiphenyl by synthesizing it from the known 3-amino-4,4′-dinitrobiphenyl.[2] The nitration of either 3-bromobiphenyl or of 3-bromo-4′-nitrobiphenyl with ethyl nitrate yields 3-bromo-4,4′-dinitrobiphenyl (I), whose structure is similarly proved. It was not found possible to isolate any of the expected 3-bromo-4′,6-dinitrobiphenyl (II) from either of these reaction mixtures. This product was synthesized, however, by the following method: 3-acetamino-4′-nitrobiphenyl[2] was converted into 3-acetamino-4′,6-dinitrobiphenyl (III) by nitration with ethyl nitrate in sulfuric acid. Hydrolysis afforded the base, which was then converted into the bromodinitro derivative. The structure of III and hence also of II was proved by the fact that on reduction and acetylation it yielded the same acetyl derivative IV as was obtained by similarly treating 2-acetamino-4′,5-dinitrobiphenyl.[3]

Analysis and molecular weight determination showed this to be a hexa-acetyl derivative. 3-Bromo-4′,6-dinitrobiphenyl also was obtained by brominating 4-nitro-4′-acetaminobiphenyl,[4] hydrolyzing, nitrating the free base, and deaminizing. This product proved to be identical with II, thus proving the structure of the bromodinitro base V.

In another attempt to synthesize II, 2-nitro-5-bromobiphenyl[4a] (m. p, 55–56°) was synthesized from 2-nitro-5-bromoaniline by Gomberg's reaction. Subsequent nitration did not, however, yield any definite product.

In the nitration of 3-bromo-4′-nitrobiphenyl with nitric and sulfuric acids, a small amount of a

(1) Mascarelli and Gatti, *Gazz. chim. ital.*, **63**, 654 (1933).

(2) Case, THIS JOURNAL, **61**, 767 (1939).

(3) Scarborough and Waters, *J. Chem. Soc.*, 89 (1927).

(4) Case, THIS JOURNAL, **60**, 424 (1938).

(4a) The compound reported by Campbell, Anderson and Gilmore, *J. Chem. Soc.*, 449 (1940), prepared from 2-amino-5-bromobiphenyl and melting at 230°, is evidently not 2-nitro-5-bromobiphenyl.