

tion with ether and water, respectively. The remainder of the products consisted of a black oil (1.5 g.) and a dark brown, high melting solid (7.5 g.). The latter when refluxed with concentrated hydrochloric acid for 4 hours gave a crystalline brown solid from which *p*-toluenesulfonamide (0.5 g.) could be recovered by extracting with boiling water. The residue from this extraction contained 11.56% sulfur.

Anal. Calcd. for $C_7H_5SO_2N$: S, 18.7. Calcd. for $C_8H_8SO_2N$: S, 17.6.

7. *pH* Measurements.—A solution of trimethylamine-

p-toluenesulfonimide (0.00419 *M*) in conductivity water gave, under nitrogen, an initial *pH* measurement of 6.72. After standing for 15 hours the reading increased to 7.90. The solution upon evaporation to dryness gave the original material. K_b for the aminimide taking the ionization of water into account is given by the expression

$$K_b(H^+)^3 + (K_bC_B + K_w)(H^+)^2 - K_wK_b(H^+) - K_w^2 = 0$$

$$K_b = 1.51 \times 10^{-10}; \text{dissociation} = 0.061\%$$

IOWA CITY, IOWA

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF IOWA STATE COLLEGE]

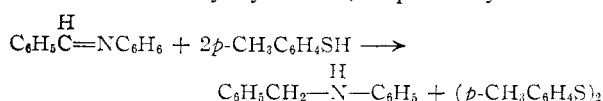
Reduction of Some Conjugated Azomethine Systems by Aryl Thiols

BY HENRY GILMAN, J. LEWIS TOWLE AND ROBERT K. INGHAM

RECEIVED JANUARY 8, 1954

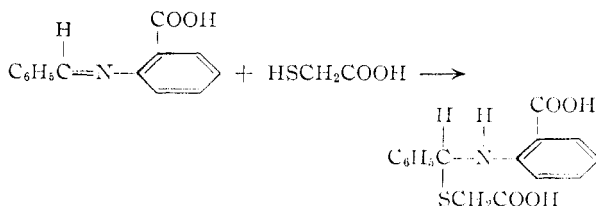
p-Thiocresol was found to be without effect on cyclic anils such as pyridine, quinoline, isoquinoline and benzothiazole. However, acridine was reduced to biacridan and acridan by this thiol. An interesting reaction with aryl thiols is the reduction of the carbon-carbon double bond in benzalquinaldine and benzallepidine to form 2-(β -phenylethyl)-quinoline and 4-(β -phenylethyl)-quinoline, respectively.

It has been shown¹ that *p*-thiocresol has a reducing action on the azomethine grouping in benzalaniline and also in benzophenone-anil, forming benzylaniline and benzhydrylaniline, respectively

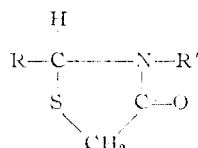


In a similar reaction with *p*-thiocresol, azobenzene was converted to hydrazobenzene.¹

More recently, Stacy and Morath² have found that β -thionaphthol did not react with benzalaniline, and that benzalanthranilic acid, instead of being reduced, formed addition products with *p*-thiocresol, thiophenol and a number of thiols in the alkyl series.



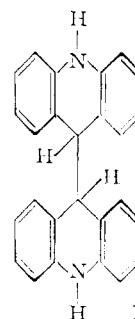
Frequently, when employing an ester of thioglycolic acid or the free acid, the addition to the azomethine grouping is followed by intramolecular condensation to give a 2,3-disubstituted-4-thiazolidone.^{3,4}



The unique reduction of benzalaniline and benzophenone-anil with *p*-thiocresol suggested the possibility of using thiols as preferential reductants for the azomethine grouping where the latter is part

of a ring system, as in quinoline or in pyridine. From the results of a number of attempted reductions with a variety of anils (see Table I), it appears that the reaction is not generally applicable to this class of compounds.

Pyridine, quinoline, isoquinoline, 2-phenylquinoline, 2-(*p*-dimethylaminophenyl)-7-methylquinoline and benzothiazole were not acted upon by *p*-thiocresol under our experimental conditions. Acridine was found to undergo reduction to biacridan (I), and in one experiment acridan was isolated as one of the products. The yields varied appreciably with the conditions employed. That steric factors may be significant in this type of reaction was indicated when 9-(*o*-iodophenyl)-acridine⁵ was recovered in quantitative amount in one attempted reduction.



The net effect of the reduction of acridine to acridan is the addition of two atoms of hydrogen to the terminal atoms of a conjugated azomethine system. The azomethine grouping is important in this reaction; anthracene, with a similar conjugated system but lacking the azomethine grouping, is not reduced under our conditions (although certain other methods of reduction are successful for both molecules).

The success of this reaction with acridine led to the investigation of several other compounds containing a conjugated azomethine system. It was

(5) The authors are indebted to Dr. C. Gardner Swain for a supply of 9-(*o*-iodophenyl)-acridine.

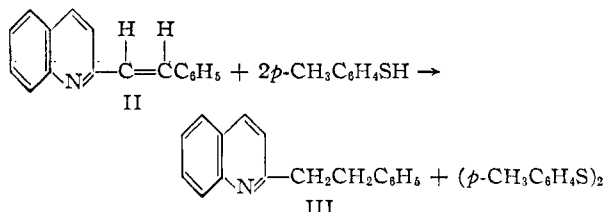
- (1) H. Gilman and J. B. Dickey, *THIS JOURNAL*, **52**, 4573 (1930).
- (2) G. W. Stacy and R. J. Morath, *ibid.*, **74**, 3885 (1932).
- (3) A. R. Surrey, *ibid.*, **69**, 2911 (1947).
- (4) H. D. Troutman and L. M. Long, *ibid.*, **70**, 3436 (1948).

TABLE I
 REDUCTIONS WITH *p*-THIOCRESOL AND THIOPHENOL

Expt. no.	Compound	Cpd., mole	Thiol, ^a mole	Reaction medium	Temp., °C.	Time of reacn., hr.	Products (yield, %)	Recovery of thiol, %
1	Pyridine	0.105	0.315	Xylene	Reflux	25	None	92.4
2	Quinoline	.105	.315	Xylene	Reflux	25	None	97.5
3	Quinoline	.105	.210	No solvent	200	24	None	93.0
4	Isoquinoline	.105	.315	No solvent	200	25	None	95.0
5	2-Phenylquinoline	.105	.315	No solvent	200	25	None	97.4
6	2-(<i>p</i> -Dimethylaminophenyl)-7-methylquinoline	.053	.158	No solvent	200	25	None	96.0
7	Benzothiazole	.114	.484	No solvent	190	36	None	98.0
8	Anthracene	.10	.23	Xylene	Reflux	24	None	93.0
9	Acridine	.105	.315	Xylene	Reflux	25	Biacridan (32.0)	50.0
10	Acridine	.10	.100	Xylene	Reflux	25	Biacridan (44.6)	12.1
11	Acridine	.210	.630	Xylene	Reflux	36	Biacridan (50.0)	49.0
12	Acridine	.105	.315	No solvent	170–180	20	Biacridan (70.1) Acridan (21.0)	46.2
13	Acridine	.20	.20	No solvent	190–200	24	Biacridan (70.0)	30.0
14	9-(<i>o</i> -Iodophenyl)-acridine	.0026	.04	Excess thiol	200	24	None	..
15	Benzalquinaldine	.0865	.205	No solvent	200–215	24	2-(β -Phenylethyl)-quinoline (86.5) ^b	11.0
16	Benzalquinaldine	.022	.045 ^a	No solvent	190–200	24	2-(β -Phenylethyl)-quinoline (80) ^b	..
17	Benzallepidine	.065	.153	No solvent	190–210	23	4-(β -Phenylethyl)-quinoline	..

^a *p*-Thiocresol was used except with expt. 16, where thiophenol was employed. ^b Weighed as the hydrochloride.

found that benzalquinaldine (II) is reduced to 2-(β -phenylethyl)-quinoline (III). Likewise, 4-(β -phenylethyl)-quinoline is obtained from benzallepidine. In each of these reactions di-*p*-tolyl disulfide was isolated as the oxidation product.



The azomethine grouping seems to be essential for the reduction of the carbon-carbon double bond in benzalquinaldine and benzallepidine, since ordinarily⁶ thiols react with olefins to form simple adducts.^{7,8} Styrene, even under forcing conditions with an excess of *p*-thiocresol, formed a sulfide, whereas stilbene, under the same conditions, failed to undergo either reduction or addition.⁹

Mercaptans add to double bonds in accordance with Markownikoff's rule if peroxides are absent or if sulfur is used as a catalyst. In the presence of peroxides, abnormal addition takes place.^{10,11} Thiophenol or *p*-thiocresol have been reported^{12,13}

to add to unsaturates contrary to Markownikoff's rule; however, if freshly distilled thiophenol or *p*-thiocresol were employed, the mode of addition was reversed,¹⁰ presumably due to the elimination of traces of peroxides.

Among other olefins which give sulfide addition products with thiols are α,β -unsaturated ketones,⁷ α -alkylacrylonitriles,¹⁴ β -alkylacrylonitriles¹⁵ and 1-cyano-1-cyclohexene.¹⁶ It is significant that each of these types has an unsaturated grouping, *i.e.*, cyano or carbonyl, conjugated with the carbon-carbon double bond as the azomethine grouping is conjugated in benzalquinaldine or in benzallepidine, and yet is not reduced by thiols.

Experimental¹⁷

Attempted Reduction of Pyridine, Quinoline, Isoquinoline, 2-Phenylquinoline, 2-(*p*-Dimethylaminophenyl)-7-methylquinoline, Benzothiazole and Anthracene with *p*-Thiocresol (see Table I, Expts. 1–8).—The experiment described below is typical for each of the above attempted reductions.

The compound and *p*-thiocresol were dissolved in 200 ml. of xylene and refluxed for 24 hours or more under a nitrogen atmosphere in a flask equipped with a trap¹⁸ to exclude air. After the solution was cooled to room temperature, it was extracted with 10% sodium hydroxide. Acidification and cooling of the alkaline extract in an ice-bath caused *p*-thiocresol to separate as large, easily filterable crystals.

The xylene was distilled and a portion of the remaining liquid, which was unreacted amine, was converted to the picrate. The melting point agreed in every case with the reported value, and its sharpness indicated the absence of possible contaminants such as dihydro compounds.

In expts. 3 and 5, the reactants were heated together without a solvent, and again there was no reduction and an excellent recovery of thiol was obtained.

The procedure was modified somewhat in working up

(6) The preparation of saturated hydrocarbons from olefins and hydrogen sulfide has been reported by E. C. Williams and C. C. Allen, U. S. Patent 2,052,268 (Aug. 25, 1936) [C. A., **30**, 7122 (1936)]. These authors suggest as a possible mechanism, the reaction of the mercaptan, formed as the result of the addition of hydrogen sulfide to the olefin, with unreacted olefin to give the saturated hydrocarbon and disulfide.

(7) T. Posner, *Ber.*, **35**, 799 (1902).

(8) B. H. Nicolet, *THIS JOURNAL*, **53**, 3066 (1931).

(9) J. L. Towle, unpublished work.

(10) S. O. Jones and E. E. Reid, *THIS JOURNAL*, **60**, 2452 (1938).

(11) M. S. Kharasch, A. T. Read and F. R. Mayo, *Chemistry & Industry*, 752 (1938).

(12) T. Posner, *Ber.*, **38**, 646 (1905).

(13) B. H. Nicolet, *THIS JOURNAL*, **57**, 1098 (1935).

(14) R. M. Ross, H. L. Bushey and R. J. Rolih, *ibid.*, **73**, 540 (1951).

(15) R. M. Ross, *ibid.*, **71**, 3458 (1949).

(16) R. M. Ross and F. W. Raths, *ibid.*, **73**, 129 (1951).

(17) All melting points are uncorrected.

(18) H. Gilman and A. P. Hewlett, *Rec. trav. chim.*, **48**, 1124 (1929).

expts. 6 and 8 because the 2-(*p*-dimethylaminophenyl)-7-methylquinoline or anthracene crystallized out of the cooled xylene solution. These were first filtered off and washed with xylene, and then the combined filtrate and washings treated as described above.

To make certain that dihydroanthracene was not formed, the recovered anthracene was subjected to a steam distillation. The dihydro compound, being volatile with steam, would have thus been separated from the starting material.

Reduction of Acridine to Biacridan (Expt. 9).—The reactants were dissolved in 200 ml. of solvent and refluxed. During the reflux period a crystalline substance continually separated. This was filtered off and washed in turn with xylene and hot ethanol. Because of the extreme insolubility of the product, attempts at purification through recrystallization did not lead to satisfactory results. The results of elemental analyses and a melting point determination (m.p. 260–265° in a metal melting point block) suggested that this compound might be the biacridan described by Schlenk and Bergmann¹⁹ and also by Bergmann and Blum-Bergmann,²⁰ similarly reported to melt at 260–265°.

Anal. Calcd. for $C_{28}H_{20}N_2$: C, 86.66; H, 5.59; N, 7.42. Found: C, 86.40, 86.72; H, 5.42, 5.47; N, 7.45.

A mixed melting point determination with a sample of Bergmann's compound²¹ failed to establish clearly whether the compounds were identical. By heating the bath to 230° and then inserting the thermometer and samples, the following melting points were obtained: the compound prepared by the thiol reduction, m.p. 255–260°; Bergmann's biacridan, m.p. 245–249°; an equal mixture of the two compounds, m.p. 240–250°.

When the thermometer and samples were inserted in the bath at room temperature, the melting points were as follows: the compound obtained by thiol reduction, m.p. 247–255°; Bergmann's compound, m.p. 225–235°; a mixture of the two compounds, m.p. 230–240°. Apparently the melting point is greatly influenced by the method used.

The failure to obtain an appreciable depression in melting point recalls the work of Lehmstedt and Hundertmark,²² whose method of preheating the bath is described above. It was found that the compound (m.p. 214°), which was believed to be acridan, and isomeric with Bergmann's compound (m.p. 249° by the method under consideration), failed to depress the melting point of the latter. It was postulated that the compound melting at 214° may have disproportionated at this temperature, and the melt which resulted, consisting of acridine and acridan, served as a non-dissolving medium for the higher melting isomer.²²

Debye powder diagrams²³ of the compound prepared by thiol reduction and that of Bergmann are identical. Lehmstedt and Hundertmark also state that their biacridan gives the same pattern as Bergmann's product.

The compound was finally identified by preparing the dibenzoyl derivative. A mixed melting point determination with a sample of the compound obtained by Schlenk and Bergmann¹⁹ from the benzoylation of the sodium-adduct of acridine demonstrated the two to be identical.

The original filtrate from which biacridan was separated was extracted with sodium hydroxide to recover unreacted thiol.

After standing over sodium sulfate, the xylene solution was evaporated to dryness, leaving a crystalline residue. This was dissolved in ethanol and filtered. A small amount of unidentified yellowish material melting above 300° remained on the filter. The ethanolic filtrate was completely evaporated and the residue taken up in anhydrous ether. Ethanolic hydrochloric acid was added to precipitate the acridine hydrochloride. This was removed by filtration and converted to the free base by titration with aqueous sodium hydroxide. The dried material weighed 11.0 g. or 58% of the original amount.

After removal of the alcohol by evaporation, the residue, weighing 8.5 g. (0.0345 mole), was recrystallized from dilute alcohol. It was shown to be di-*p*-tolyl disulfide by comparison with an authentic sample.

9,9'-Dibenzoylbicridan.—To 0.04 mole of phenyllithium²⁴ in an ether-xylene solution was added 3.46 g. (0.0095 mole) of biacridan, and the mixture was refluxed for 24 hours. An excess of benzoyl chloride was then added and the refluxing continued for an additional 6 hours. The insoluble material was filtered off and washed thoroughly with acetone. The crude product melted over the range 270–290°. After recrystallization from cumene, the melting point was raised to 305°. A mixture of Bergmann's 9,9'-dibenzoylbicridan¹⁹ and our preparation melted at 303–304°.

9-(*o*-Iodophenyl)-acridine and *p*-Thiocresol (Expt. 14).—After heating the reactants in the usual manner, 200 ml. of petroleum ether (b.p. 60–70°) was added to the cooled reaction mixture, whereby complete solution was effected. Cooling resulted in the separation of crystals. These were filtered off, washed with cold petroleum ether (b.p. 28–38°) and dried in a desiccator. The product weighed 0.95 g. and melted at 258°. A mixture of this material with 9-(*o*-iodophenyl)-acridine (m.p. 262°) melted at 261–262°, and indicated that the starting material was recovered in a practically quantitative amount.

Acridan from the Reduction of Acridine (Expt. 12).—The reactants were heated as above, and to the cooled reaction mixture was added 300 ml. of ether; the crystalline mass was easily broken up with a spatula. The product was filtered off and washed successively with hot absolute ethanol and ether. This solid melted at 260–265° and was identified as biacridan.

Unreacted thiol was recovered by extracting the ethereal solution with sodium hydroxide and acidifying the cooled extract.

After drying the ether solution over sodium sulfate and then evaporating to dryness, a residue was obtained which was transferred to a sintered glass funnel and washed quickly with petroleum ether (b.p. 28–38°). This treatment dissolved everything except the di-*p*-tolyl disulfide, which weighed 16.0 g. (0.065 mole). Evaporation of the ether from the filtrate left a mixture of acridine and acridan weighing 5.2 g. These two compounds were separated by means of dilute ethanolic hydrochloric acid. The insoluble acridan, weighing 4.0 g. (0.022 mole), was identified by a mixed melting point with an authentic specimen prepared by the sodium amalgam reduction of acridine.²⁵ In expt. 13, using equimolecular amounts of thiol and acridine, the same yield of biacridan was obtained but no acridan was isolated.

Benzalquinaldine²⁶ and *p*-Thiocresol (Expt. 15).—The cold reaction mixture was dissolved in 100 ml. of ether and then extracted with 10% hydroxide. The amount of recovered thiol (0.024 mole) was approximately equal to the quantity of thiol which was added in excess of the theoretical amount.

The ethereal solution was dried over sodium sulfate and then saturated with dry hydrogen chloride. By this treatment the hydrochloride of 2-(*β*-phenylethyl)-quinoline separated as a red gum, leaving the second product, di-*p*-tolyl disulfide, in solution. Decantation of the solvent followed by evaporation, left a residue which was shown to be di-*p*-tolyl disulfide by the method of mixed melting point determination.

The gummy hydrochloride was dissolved in ethanol and treated with picric acid. The yellow crystalline precipitate was identified as the picrate of 2-(*β*-phenylethyl)-quinoline by comparison with an authentic specimen.²⁷

If anhydrous benzene is used as a solvent instead of ether, the hydrochloride can be obtained in a crystalline form at ice-bath temperature.

From a similar reaction of benzalquinaldine with thiophenol (expt. 16), 2-(*β*-phenylethyl)-quinoline was obtained and isolated as the hydrochloride. Identification was accomplished through the picrate (m.p. 134–135°).

Benzallepidine²⁸ and *p*-Thiocresol (Expt. 17).—The cooled reaction mixture was dissolved in benzene, extracted with sodium hydroxide to remove excess thiol, dried over sodium

(19) W. Schlenk and E. Bergmann, *Ann.*, **463**, 300 (1928).

(20) E. Bergmann and O. Blum-Bergmann, *Ber.*, **63**, 757 (1930).

(21) The authors are grateful to Dr. Ernst Bergmann for samples of biacridan and 9,9'-dibenzoylbicridan.

(22) K. Lehmstedt and L. Hundertmark, *Ber.*, **63**, 1229 (1930).

(23) The authors are indebted to Dr. Richard Raeuchle for the Debye powder diagrams.

(24) R. G. Jones and H. Gilman, "Organic Reactions," Vol. VI, John Wiley and Sons, Inc., New York, N. Y., 1951, p. 353.

(25) C. Graebe and H. Caro, *Ann.*, **158**, 265 (1871).

(26) S. Skraup and K. Böhm, *Ber.*, **59**, 1013 (1926).

(27) K. Ziegler and H. Zeiser, *Ann.*, **485**, 174 (1931).

(28) C. E. Kaslow and K. D. Stayner, *THIS JOURNAL*, **67**, 1716 (1945).

sulfate and then saturated with gaseous hydrogen chloride. The hydrochloride which separated was filtered off, washed with benzene, dissolved in 95% ethanol and neutralized with dilute ammonium hydroxide. The addition of an excess of water caused an oil to separate. This oil was dissolved in hot dilute ethanol, and the resulting solution was cooled. The white crystals which separated melted at 101–103°.

and did not depress the melting point of an authentic specimen of 4-(β -phenylethyl)-quinoline.²⁹

Acknowledgment.—The authors are grateful to Howard A. Hartzfeld for assistance.

(29) B. Heymann and W. Koenigs, *Ber.*, **21**, 1424 (1888).

AMES, IOWA

[CONTRIBUTION NO. 1868 FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY]

Stepwise Dehydrogenation of the Colorless Polyenes Phytoene and Phytofluene with N-Bromosuccinimide to Carotenoid Pigments

BY L. ZECHMEISTER AND B. KENNETH KOE

RECEIVED JANUARY 11, 1954

Dehydrogenation of the colorless plant polyenes phytoene and phytofluene can be carried out with N-bromosuccinimide and some other reagents. This stepwise process takes place mainly at the ends of the conjugated system and leads through the following series: phytoene \rightarrow phytofluene \rightarrow ζ -carotene \rightarrow neurosporene \rightarrow lycopene. The artifacts were identified with the corresponding natural products. The exact mechanism of some of the steps mentioned is unknown.

The representatives of the naturally occurring C_{40} -polyenes with isoprenic structure belong either to the subclass of colorless, fluorescent compounds such as phytoene¹ and phytofluene² or to the carotenoid pigments proper containing longer conjugated systems that extend over 7 to 15 conjugated double bonds. As reported briefly,³ a transition from the first to the second type can be achieved by dehydrogenation *in vitro*. So far as we know the most convenient agent for this purpose is N-bromosuccinimide (NBS); various other compounds such as N-bromoacetamide (NBA), *p*-benzoquinone, diphenylquinone, isatin and *o*-nitrosonitrobenzene, can also be applied in principle but the yields are very low in the latter three instances.

In the present study the experimental conditions of the following dehydrogenation steps are described: phytoene (3 conj. double bonds) \rightarrow phytofluene (5) \rightarrow ζ -carotene⁴ (7) \rightarrow neurosporene⁵ (9) \rightarrow lycopene, $C_{40}H_{56}$ (11). After having treated any of these polyenes with N-bromosuccinimide, a subsequent chromatographic resolution shows the presence of all those members of the above series that are more unsaturated than the compound treated. The main product is in each instance that polyene whose conjugated system contains two more double bonds than the starting material. However, neurosporene forms lycopene in very poor yields under the conditions applied. In contrast, according to Karrer and Rutschmann,⁶ the further dehydrogenation of lycopene gives sub-

stantial amounts of dehydrolycopene ($C_{40}H_{52}$, 15 conj. double bonds), possibly because in that particular instance the two newly formed double bonds establish connection by conjugation of the main chromophore with the two formerly isolated double bonds located in the terminal isopropylidene groups of the lycopene molecule.

In the course of a rather extended study of the pertinent experimental conditions the following observations were made. Although the reaction involves liberation of hydrogen bromide, brominated compounds have not been encountered. Partial destruction of the conjugated system did take place in every instance but its extent could be limited by using not more than 1 mole reagent per mole polyene. It is advantageous to carry out such conversions in the presence of glacial acetic acid⁷ whereby the dehydrogenation proceeds rapidly. Thus, upon treatment of practically non-fluorescent phytoene the strong fluorescence of newly formed phytofluene appeared within half a minute; and starting from phytofluene, the mixture turned a dark red in a minute or so. These two conversions require moderate heating, but the further ones some cooling. For yields *cf.* Table I. In the conversion of phytoene to phytofluene the yields were reduced by the presence of N-phenylmorpholine, sodium ace-

TABLE I
YIELDS, ESTABLISHED PHOTOMETRICALLY, IN THE STEPWISE DEHYDROGENATION OF SOME POLYENES

Dehydrogenation step	Reagent	Yield based on starting mater., %	Yield based on converted starting mater., %
Phytoene \rightarrow phytofluene	NBS	26	40
Phytoene \rightarrow phytofluene	NBA	16	31
Phytoene \rightarrow phytofluene	Quinone	9	35
Phytofluene \rightarrow ζ -carotene	NBS	28	40
ζ -Carotene \rightarrow neurosporene	NBS	19	27
Neurosporene \rightarrow lycopene	NBS	4	7

(7) L. Bateman, J. I. Cuneen and H. P. Koch, *J. Chem. Soc.*, 8045 (1950); G. Dupont, R. Dulou and N. Defay, *Bull. soc. chim. France*, 310 (1949); R. Tschesche and F. Korte, *Ber.*, **84**, 77 (1951).

(1) J. W. Porter and F. P. Zscheile, *Arch. Biochem.*, **10**, 547 (1946); J. W. Porter and R. E. Lincoln, *ibid.*, **27**, 390 (1950); W. J. Rouborn and F. W. Quackenbush, *ibid.*, **44**, 159 (1953).

(2) L. Zechmeister and A. Polgár, *Science*, **100**, 317 (1944); L. Zechmeister and A. Sandoval, *Arch. Biochem.*, **8**, 425 (1945); *This Journal*, **68**, 197 (1946); V. Wallace and J. W. Porter, *Arch. Biochem. Biophys.*, **36**, 468 (1952); *cf.* H. H. Strain, *J. Biol. Chem.*, **127**, 191 (1939); "Leaf Xanthophylls," Carnegie Inst. of Washington, 1938.

(3) B. K. Koe and L. Zechmeister, *Arch. Biochem. Biophys.*, **41**, 236 (1952). Previously, squalene, $C_{30}H_{50}$, had been dehydrogenated to polyene pigments in our laboratory by J. Dale, *ibid.*, **41**, 475 (1952).

(4) H. A. Nash, F. W. Quackenbush and J. W. Porter, *This Journal*, **70**, 3613 (1948); H. A. Nash and F. P. Zscheile, *Arch. Biochem.*, **7**, 305 (1945); *cf.* also H. H. Strain, *ref. 2*.

(5) F. T. Haxo, *Arch. Biochem.*, **20**, 400 (1949).

(6) P. Karrer and J. Rutschmann, *Helv. Chim. Acta*, **28**, 793 (1945).