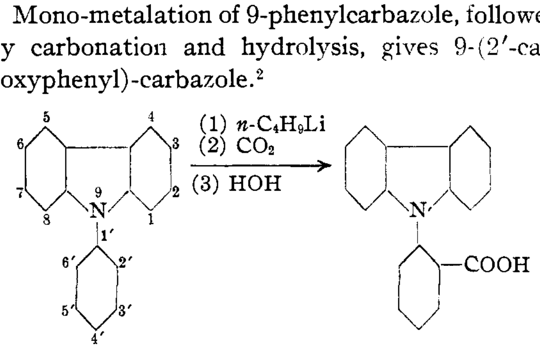
[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF IOWA STATE COLLEGE]

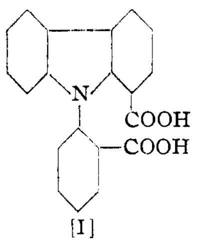
The Di-metalation of 9-Phenylcarbazole[1]

BY HENRY GILMAN AND C. G. STUCKWISCH

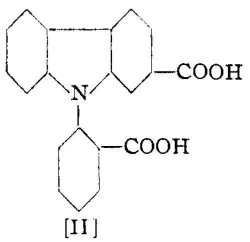
Mono-metalation of 9-phenylcarbazole, followed by carbonation and hydrolysis, gives 9-(2'-carboxyphenyl)-carbazole.[2]



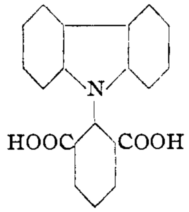
In addition to the mono-carboxylic acid, there is formed in 25% yield a di-carboxylic acid. Inasmuch as partial decarboxylation[3] of the dibasic acid gives 9-(2'-carboxyphenyl)-carbazole, one of the positions involved in di-metalation is established. Accordingly, the number of isomeric dibasic acids possible is reduced to seven, and of these the more important ones appeared to be I, II and III.



[I]



[II]



[III]

Since metalation generally takes place ortho to a hetero element,[4] and since no cases of homonuclear di-metalation have been observed,[4] compound [I] was considered most probable. However, the synthesis of an authentic specimen (see Experimental) of 9-(2'-carboxyphenyl)-carbazole-1-carboxylic acid [I] showed this compound to be unlike the dibasic acid formed as a result of the di-metalation.

The next isomeric acid considered was [II]. It seemed reasonable that one of the carboxyl groups might be in a meta-position, since triphenylamine was shown earlier to metalate unexpectedly in a meta-position.[5] The 9-(2'-carboxyphenyl)-carbazole-2-carboxylic acid was prepared by reliable reactions (see Experimental) and it also was shown to be unlike the di-metalation acid.

Subsequently two of the other possible dibasic acids were prepared (see Table I in Experimental) and shown to be unlike the dibasic acid isolated. Also the possibility of 9-(2',3'-dicarboxyphenyl)-carbazole was considered unlikely on the basis of a negative fluorescein test.

There remained accordingly, only two possible structures: 9-(2'-carboxyphenyl)-carbazole-4-carboxylic acid, and 9-(2',6'-dicarboxyphenyl)-carbazole [III]. Inasmuch as there is no convenient approach to the synthesis of the former compound, attempts were made to synthesize compound [III]. First, a condensation was tried between carbazole and dimethyl 2-iodoisophthalate. None of the expected di-ester was obtained, and the chief product obtained subsequent to hydrolysis was a 70% yield of 2,6,2',6'-tetracarboxybiphenyl. Second, a condensation was attempted between carbazole and 2-iodo-1,3-dimethylbenzene with the plan of oxidizing any resulting 9-(2',6'-dimethylphenyl)-carbazole to the corresponding dibasic acid. However, 97% of the carbazole was recovered. The absence of the expected condensation products in these two reactions is due undoubtedly to pronounced steric influences.

Although we were unsuccessful in synthesizing an authentic specimen of 9-(2',6'-dicarboxyphenyl)-carbazole [III], we did show that the di-metalation acid must have structure III. This was done indirectly by the following sequence of reactions

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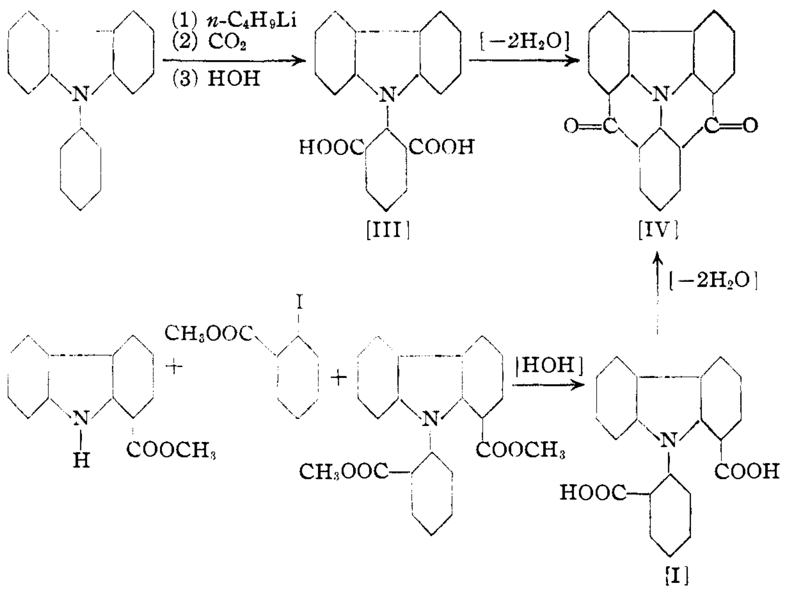
(1) Paper LIII in the series; The relative reactivities of organometallic compounds. The preceding paper is in THIS JOURNAL, **65**, 1461 (1943).

(2) Gilman, Stuckwisch and Kendall, *ibid.*, **63**, 1758 (1941).

(3) Partial decarboxylation of some polybasic acids of heterocycles, particularly in the furan series, goes preferentially and involves but one position predominantly.

(4) Gilman and Young, THIS JOURNAL, **56**, 1415 (1934); **57**, 1421 (1935); Gilman and Kirby, *J. Org. Chem.*, **1**, 146 (1936); Gilman and Bebb, THIS JOURNAL, **61**, 109 (1939); Gilman, Brown, Webb and Spatz, *ibid.*, **62**, 977 (1940); Gilman, Van Ess, Willis and Stuckwisch, *ibid.*, **62**, 2606 (1940).

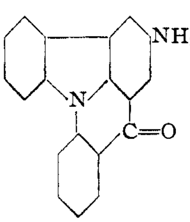
(5) Gilman and Brown, *ibid.*, **62**, 3208 (1940).



The same diketone [IV] was obtained by the cyclodehydration of dibasic acid III and of dibasic acid I; and [III] is the only dibasic acid with a 2′-carboxyphenyl group that could give a diketone identical with that given by the authentic dibasic acid I.

It is interesting to note that the steric factors which operated to some extent to frustrate the synthesis of [III] as well as the corresponding 2′,6′-dimethylphenyl compound, did not interfere to prevent the unusual di-metalation. Also in conformity with generalizations pointed out in preceding papers,[4] metalation makes available the introduction of nuclear substituents in positions otherwise inaccessible by substitution reactions like halogenation, nitration, etc.[6]

The cyclodehydration derivatives of 9-phenylcarbazole are of general interest in problems of stereochemistry. Hayashi[7] has reported the attempted resolution of 6-amino-[8-indolo(3,2,1-de)acridin-8-one] [V]. The diketone obtained by us from the cyclodehydration of 9-(2′,6′-dicarboxyphenyl)-carbazole [IV] is a more closely knit system, and more attractive from the point of view of stereochemical opportunities. Although the resonance involving the carbonyl groups would probably interfere with the resolution of an appropriately substituted derivative of [IV], it was of interest to determine whether the carbonyl groups were sufficiently reactive to undergo condensation with *l*-menthyl N-aminocarbamate.[8] This particularly suitable resolving agent for carbonyl compounds did not react with the diketone [IV].



[V]

By way of introducing an amino group that might be used in studies on resolution, the 9-(2′,6′-dicarboxyphenyl)-carbazole was nitrated. In the first experiments it was found that a dinitro compound was the chief product. Decarboxylation of this dinitrodibasic acid gave a dinitro-9-phenylcarbazole. This compound was shown to be 3,6-dinitro-9-phenylcarbazole by comparison with an authentic specimen prepared by the condensation of 3,6-dinitrocarbazole with iodobenzene.

The mono-nitration of 9-(2′,6′-dicarboxyphenyl)-carbazole, effected under milder conditions, gave 3-nitro-9-(2′,6′-dicarboxyphenyl)-carbazole. The structure of this compound was established by the same general procedures used with the corresponding dinitro compound. In an orienting experiment, it was observed that the nitro group interferes with the otherwise facile cyclodehydration noted with the nitro-free dibasic acid.

Experimental

Metalation of 9-Phenylcarbazole.—A mixture of 20 g. (0.082 mole) of 9-phenylcarbazole and 0.25 mole of *n*-butyllithium in 250 ml. of ether was stirred and refluxed for forty-eight hours, and then carbonated by pouring on crushed solid carbon dioxide. Acidification of the alkaline extract gave 10.4 g. of acidic material. From the benzene insoluble portion of the acidic material was isolated 6 g. or 25% of 9-(2′,6′-dicarboxyphenyl)-carbazole [III]. After several crystallizations from 80% ethanol the melting point of the acid was 273–274°.

Anal. Calcd. for $C_{20}H_{13}O_4N$: N, 4.32; neut. equiv., 165. Found: N, 4.41; neut. equiv., 166.5.

Decarboxylation of 9-(2′,6′-dicarboxyphenyl)-carbazole by heating to 280° gave 87% of 9-(2′-carboxyphenyl)-carbazole.

9-(2′,6′-Di-carbomethoxyphenyl)-carbazole.—The dimethyl ester was prepared in 95% yield from the acid and diazomethane. After crystallization from methanol the ester melted at 156–157°.

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[6] Unpublished studies by Fred Lister on nuclear substitution reactions of 9-phenylcarbazole.

[7] Hayashi, *Bull. Inst. Phys.-Chem. Research* (Tokyo), **9**, 970 (1930) [*C. A.*, **25**, 2997 (1931)].

[8] Woodward, Kohman and Harris, THIS JOURNAL, **63**, 120 (1941).

TABLE I

SOME DICARBOXYLIC ACIDS AND THEIR DIMETHYL ESTERS OF 9-PHENYLCARBAZOLE

Compound	M. p., °C.	Yield, %	Analyses, %			
			Nitrogen		Neut. equiv.	
			Calcd.	Found	Calcd.	Found
9-(2'-Carboxyphenyl)-carbazole-1-carboxylic acid	231–232	28	4.32	4.40	165	162
9-(2'-Carboxyphenyl)-carbazole-1-carboxylic acid dimethyl ester	144–145	90	3.90	3.81		
9-(2'-Carboxyphenyl)-carbazole-2-carboxylic acid[a]	266–267	31	4.32	4.26	165	168
9-(2'-Carboxyphenyl)-carbazole-2-carboxylic acid dimethyl ester	146–147	95	3.90	3.72		
9-(2'-Carboxyphenyl)-carbazole-3-carboxylic acid[b]	246–247	34	4.32	4.25	165	163
9-(2'-Carboxyphenyl)-carbazole-3-carboxylic acid dimethyl ester	143–144	92	3.90	4.12		
9-(2',4'-Dicarboxyphenyl)-carbazole[c]	278–280	48	4.32	4.23	165	167
9-(2',4'-Dicarboxyphenyl)-carbazole dimethyl ester	160–161	97	3.9	3.78		

[a] Carbazole-2-carboxylic acid was prepared in essential accordance with the procedure of Borsche and Feise, *Ber.*, **40**, 378 (1907), by the alkali fusion of 2,9-diacetylcarbazole. [b] Carbazole-3-carboxylic acid was kindly supplied by S. M. Spatz; *cf.* Gilman and Spatz, THIS JOURNAL, **63**, 1553 (1941). [c] This compound was prepared from carbazole and dimethyl 4-iodoisophthalate. 4-Iodoisophthalic acid was prepared by the permanganate oxidation of 4-iodo-1,3-dimethylbenzene [Grahl, *Ber.*, **28**, 89 (1895)]. The latter compound was prepared by the iodination of *m*-xylene in essential accordance with the procedure of Vorma and Raman, *J. Ind. Chem. Soc.*, 12, 343 (1935).

Anal. Calcd. for $C_{22}H_{17}O_4N$: N, 3.9. Found: N, 3.81.

Benzo[ij]carbazolo[1,9,8-cdef]quinolizine-7,11-dione [IV].—A mixture of 3.3 g. (0.01 mole) of 9-(2',6'-dicarboxyphenyl)-carbazole and 8 g. (0.04 mole) of phosphorus pentachloride was allowed to react in 25 ml. of xylene at room temperature for twenty minutes. The solution was then cooled to 0° and 6 ml. of anhydrous stannic chloride was added.[9] The solution was allowed to stand ten minutes and then hydrolyzed with iced hydrochloric acid. The xylene layer was washed successively with 10% hydrochloric acid, 10% potassium hydroxide, and water. The aqueous layer from the mixture was filtered and the residue was extracted with hot xylene. This extract was combined with the xylene layer from the original reaction mixture. Upon standing, the solution deposited irregular light-yellow crystals of the diketone. After several crystallizations from xylene the compound melted at 228–230°. The yield was 2.5 g. or 80%.

Anal. Calcd. for $C_{20}H_9O_2N$: N, 4.77. Found: N, 4.90.

Monoxime of Benzo[ij]carbazolo[1,9,8-cdef]quinolizine-7,11-dione.—A mixture of 0.6 g. (0.002 mole) of the diketone, 20 ml. of a sodium hydroxide–ethanol solution (10 g. of sodium hydroxide in 20 ml. of water and 140 ml. of ethanol) and 6 ml. of a hydroxylamine solution (7.25 g. of hydroxylamine hydrochloride in 9 ml. of water and 140 ml. of ethanol) was refluxed for fifteen hours. The mixture was then diluted with 100 ml. of water and filtered. The filtrate was acidified with 10% hydrochloric acid to precipitate the oxime. After several crystallizations from dilute ethanol the oxime melted at 262–264°. A nitrogen analysis showed that the compound was the monoxime.

Anal. Calcd. for $C_{20}H_{10}O_2N_2$: N, 9.06. Found: N, 9.09.

Carbazole-1-carboxylic Acid.—Carbazole-1-carboxylic acid was prepared in essential accordance with the procedure of Oddo,[10] by the high-temperature carbonation of 9-carbazolylmagnesium bromide. The yield of crude acid was 27%. The yield of pure product, crystallized from glacial acetic acid, was 18% and the melting point was 275–276°. The acid was converted to the methyl ester by the action of methanol in the presence of concd. sulfuric acid. After crystallization from methanol, the yield of the ester, melting at 98–100°, was 88%.

9-(2'-Carboxyphenyl)-carbazole-1-carboxylic Acid.—[I].—A mixture of 3 g. (0.0133 mole) of 1-carbomethoxycarbazole, 3 g. (0.011 mole) of methyl *o*-iodobenzoate, 3 g. of potassium carbonate and 0.5 g. of copper bronze in 10 ml. of nitrobenzene was stirred and refluxed for twelve hours. The nitrobenzene was removed by steam distillation. To facilitate isolation of the desired product, the residue was hydrolyzed by refluxing for one hour with 30% potassium hydroxide. After filtration and decolorization of the filtrate with Norite, the acid was precipitated with concd. hydrochloric acid. After several crystallizations from 50% ethanol, the acid melted at 231–232°. The yield was 28%.

Anal. Calcd. for $C_{20}H_{13}O_4N$: N, 4.32; neut. equiv., 165. Found: N, 4.40; neut. equiv., 168.

The dimethyl ester of 9-(2'-carboxyphenyl)-carbazole-1-carboxylic acid was prepared from the acid and diazo methane. After crystallization from methanol the ester melted at 144–145°.

Anal. Calcd. for $C_{22}H_{17}O_4N$: N, 3.90. Found: N, 3.81.

The dehydration of 9-(2'-carboxyphenyl)-carbazole-1-carboxylic acid was carried out as described for the metalation-acid. The yield was 60% and the melting point, 226–

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[9] Fieser and Fieser, THIS JOURNAL, **57**, 782 (1935).

[10] Oddo, *Gazz. chim. ital.*, **41**, 255 (1911).

227°. A mixed melting point determination with the diketone obtained from the acid obtained by metalation showed no depression.

Dicarboxylic Acids of 9-Phenylcarbazole.—All of the dicarboxylic acids of 9-phenylcarbazole described in this paper were prepared by the condensation of the properly substituted carbazole compound with the appropriately substituted carboxylic acid ester of iodobenzene. The general procedure was similar to that described for the preparation of 9-(2′-carboxyphenyl)-1-carboxylic acid. The dimethyl esters were prepared from the acids and diazomethane. The results are summarized in Table I.

2-Iodoisophthalic Acid.—2-Iodoisophthalic acid was prepared by the oxidation of 2-iodo-1,3-dimethylbenzene. Ten grams (0.043 mole) of 2-iodo-1,3-dimethylbenzene and 20 g. of potassium permanganate in 500 ml. of water were stirred and refluxed for twenty hours. The hot solution was filtered and the filtrate was acidified with concd. hydrochloric acid, while hot. Upon cooling, the solution deposited crystals of 2-iodoisophthalic acid. The yield was 6.2 g. or 60%. The acid melted at 260° with decomposition. The melting points previously reported are 205–220°[11] and 236°.[12] The acid was analyzed for iodine and a neutral equivalent was taken. Both showed that the correct compound had been obtained.

Anal. Calcd. for $C_8H_5O_4I$: I, 43.49; neut. equiv., 146. Found: I, 43.52; neut. equiv., 148.

Attempted Preparation of 9-(2′,6′-Dicarboxyphenyl)-carbazole.—The attempted condensation of dimethyl 2-iodoisophthalate and carbazole according to the general procedure described earlier, failed to give the desired acid. Instead a 70% yield of 2,6,2′,6′-tetracarboxyphenyl melting at 390° (dec.)[13] was obtained subsequent to hydrolysis.

Attempted Preparation of 9-(2′,6′-Dimethylphenyl)-carbazole.—A mixture of 10 g. (0.043 mole) of 2-iodo-1,3-dimethylbenzene, 7 g. (0.042 mole) of carbazole, 10 g. of potassium carbonate and 1 g. of copper bronze in 20 ml. of nitrobenzene was stirred and refluxed for twelve hours. Steam distillation of the mixture removed the nitrobenzene and unreacted 2-iodo-1,3-dimethylbenzene. The residue was crystallized from 95% ethanol. A melting point and mixed melting point showed that this was carbazole; 97% of the carbazole was recovered.

Reaction of 9-(2′,6′-Dilithiophenyl)-carbazole with Dimethyl Sulfate.—During the progress of this investigation it was hoped that 2-iodo-1,3-dimethylbenzene might be condensed with carbazole and the resulting compound compared with the dimethyl compound obtained by the reaction of dimethyl sulfate on the dimetalation product of 9-phenylcarbazole.

A mixture of 0.062 mole of *n*-butyllithium and 6 g. (0.02 mole) of 9-phenylcarbazole in 150 ml. of ether was stirred and refluxed for forty-eight hours and then treated dropwise with 0.06 mole of dimethyl sulfate in 25 ml. of ether. The mixture was allowed to stand for five hours and was then decanted through a filter. The ether was evaporated and the residue was subjected to crystallization from ethanol. Since no separation from the unreacted 9-phenylcarbazole could be accomplished in this manner, the mixture was distilled at 4 mm. This, however, also failed to effect a separation.

Di-nitration of 9-(2′,6′-Dicarboxyphenyl)-carbazole.—To 4.6 g. (0.02 mole) of 9-(2′,6′-dicarboxyphenyl)-carbazole in 30 ml. of glacial acetic acid, heated to 100° in an oil-bath, was added dropwise 4 ml. of concentrated nitric acid in 10 ml. of acetic acid. The mixture was poured into ice water. The solid which separated was crystallized from ethanol. The yield of compound melting above 350° was 4 g. or 60%. Analysis showed that it was a dinitro compound.

Anal. Calcd. for $C_{20}H_{11}N_3O_8$: N, 10.0. Found: N, 10.04.

Decarboxylation of the dinitro compound by heating between 380 and 400° for fifteen minutes gave a compound melting at 298°. This compound was shown to be 3,6-dinitro-9-phenylcarbazole by its identity with the compound obtained by the condensation of 3,6-dinitrocarbazole with iodobenzene.

Anal. Calcd. for $C_{18}H_{11}N_3O_4$: N, 12.62. Found: N, 12.68.

Mono-nitration of 9-(2′,6′-Dicarboxyphenyl)-carbazole.—To 4.6 g. (0.02 mole) of 9-(2′,6′-dicarboxyphenyl)-carbazole in 30 ml. of glacial acetic acid was added dropwise 4 ml. of concd. nitric acid in 10 ml. of acetic acid at room temperature. After thirty minutes the mixture was poured into ice water and the solid which separated was crystallized from 95% ethanol. The yield of product melting at 282–284° was 4.2 g. or 75%.

Anal. Calcd. for $C_{20}H_{12}O_6N_2$: N, 7.60. Found: N, 7.62.

Decarboxylation of the nitro compound was accomplished by heating at 350° for ten minutes. The decarboxylated compound melted at 185–187°. A mixed melting point with an authentic specimen of 3-nitro-9-phenylcarbazole[6] showed no depression.

Attempted Cyclodehydration of 3-Nitro-9-(2′,6′-dicarboxyphenyl)-carbazole.—Three and seven-tenths grams (0.01 mole) of 3-nitro-9-(2′,6′-dicarboxyphenyl)-carbazole was treated essentially in the manner described previously for the cyclodehydration of 9-(2′,6′-dicarboxyphenyl)-carbazole. The material obtained from this reaction melted between 230 and 245° and was soluble in dilute alkali. Repeated crystallization from various organic solvents failed to improve the melting point of the material.

***l*-Menthyl N-Aminocarbamate.**—*l*-Menthyl N-aminocarbamate was prepared in essential accordance with the procedure of Woodward, Kohman and Harris.[8] The yield of the compound was checked and the resolution of *dl*-camphor was likewise duplicated.

Benzo[ij]carbazolo[1,9,8-cdef]quinolizine-7-11-dione and *l*-Menthyl N-Aminocarbamate.—*l*-Menthyl N-aminocarbamate (2.03 g.) (0.01 mole) and 2.9 g. (0.01 mole) of the diketone were dissolved in 50 ml. of ethanol containing 1 g. of sodium acetate and 0.5 g. of glacial acetic acid. The mixture was stirred and refluxed for nine days. No reaction occurred during this time, since 2.6 g. of the diketone was recovered and no condensation product was isolated.

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(11) Mayer, *Ber.*, **44**, 2298 (1911).

(12) James, Kenner and Stubbings, *J. Chem. Soc.*, **117**, 773 (1920).

(13) Vollman, Beeker, Corell, Streeck and Langbein, *Ann.*, **531**, 1 (1937).

Summary

It has been shown that *n*-butyllithium dimetalates 9-phenylcarbazole homonuclearly to give, subsequent to carbonation and hydrolysis, 9-(2′,6′-dicarboxyphenyl)-carbazole. The structure of this dibasic acid was established by cyclodehydration reactions. Some experiments concerned with resolution studies of cyclodehydration derivatives are described.

AMES, IOWA RECEIVED MAY 18, 1943

[CONTRIBUTION FROM THE UNIVERSITY OF MINNESOTA AND FROM THE C. F. KETTERING FOUNDATION FOR THE STUDY OF CHLOROPHYLL AND PHOTOSYNTHESIS]

The Isolation of Photosensitizing Agents from Buckwheat[1]

BY SIMON H. WENDER,[2] ROSS A. GORTNER AND O. L. INMAN

Fagopyrism, commonly called buckwheat poisoning, was recognized as early as 1833 as being produced only in the presence of light.[3] Experimentally, Wedding[4] showed that following the ingestion of the buckwheat plant, certain white animals, such as white pigs, sheep, and cows become sensitized to the action of sunlight. These same animals, however, remain unharmed so long as they are kept out of sunlight. The general symptoms of fagopyrism consist of severe itchings, pruritus, erythema, edematous swellings of the face and ears, and, in many cases, convulsions, cerebral excitement, paralysis, and even death. The symptoms vary with the animal, and with the intensity and duration of sunlight. Blum[5] has recently presented an extensive and critical review on the reported phases of fagopyrism and related light diseases.

Oehmke,[6] Fischer,[7] Lutz and Schmid,[8] and other investigators attempted to isolate the active substances from buckwheat, but were only partially successful. Recently, Chick and Ellinger[9] found that the activating principle could be extracted from dried buckwheat flowers with mixtures of 10 parts of glacial acetic acid and 90 parts of methanol. These workers reported that studies on methods of further separation of the pigments were in progress. In this investigation, we have succeeded in adapting for use an experimental procedure for the isolation and purification of three substances from buckwheat. When each of the three substances thus isolated was fed to white guinea pigs, the animals became sensitized to sunlight.

Isolation and Studies of the Photosensitizing Agents.—The method of isolation involves extraction with proper solvents, chromatographic adsorption analysis, partition between immiscible solvents, and crystallization. Care was taken throughout the experimental work to avoid drastic chemical treatment and direct sunlight.

Five kilograms of air-dried, ground buckwheat (*Fagopyrum esculentum*) gathered when flowering, were extracted with 80% acetone, according to the method described for the preparation of chlorophyll from plants by Schertz.[10] The acetone solution was transferred to ether in a separatory funnel, and the ether washed carefully with water. The ether contained the substances desired. After removal of the ether by distillation on a steam-bath, a dark, waxy-like residue was obtained. This residue was dissolved in acetone which had been previously dried over anhydrous sodium sulfate. The acetone solution was chromatographed through a column containing talc (Merck, purified U. S. P.) tightly packed in the lower part of the column, and a smaller amount of anhydrous sodium sulfate (Merck, A. C. S. specifications) packed on top of the talc. The chromatogram was "developed" by washing with dried acetone. A blackish-purple material containing a substance termed "A" was on top of the anhydrous sodium sulfate. The talc was a light pinkish-purple color which gradually faded near the bottom into a pinkish-orange color.

Substance "A" was obtained by centrifuging with warm water the blackish-purple material from the top of the anhydrous sodium sulfate; then decanting the supernatant liquid, and repeating until the residue was freed of sodium sulfate. After drying *in vacuo*, the material was dissolved in hot, pure glacial acetic acid, filtered, and the filtrate con-

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(1) This communication contains material from a dissertation presented by Simon H. Wender to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the Ph.D. degree, June, 1938. The final manuscript was prepared after the death of Dr. Gortner and of Dr. Inman.

(2) Present address: Chemistry Department, University of Kentucky, Lexington, Kentucky.

(3) Hertwig, "Praktische Arneizmittellehre für Tierärzte," Berlin, 1833.

(4) Wedding, *Verh. d. Berl. Anthrop. Ges. Z. f. Ethnologie*, **19**, 67 (1887).

(5) Harold F. Blum, "Photodynamic Action and Diseases Caused by Light," Reinhold Publishing Corp., New York, N. Y., 1941.

(6) W. Oehmke, *Zentr. Physiol.*, **22**, 685 (1908).

(7) J. Fischer, Inaug. Dissert. Berlin, Köln-Ehrenfeld, 1909.

(8) H. E. W. Lutz and G. Schmid, *Biochem. Z.*, **226**, 67 (1930).

(9) H. Chick and P. Ellinger, *J. Physiol.*, **100**, 212 (1941).

(10) F. M. Schertz, *Plant Physiol.*, **3**, 487 (1928).