differences in method of observing absorption. Below 250 mμ discrepancies have been observed for other materials[13] between spectra obtained by different methods in common use. Considering the possibility that the discrepancy in Windaus' observed absorption[14] may be due to technique and in view of the fact that Windaus' deductions from later analyses agree with our own, we attach little weight to Heyroth and Loofbourow's suggestion that Windaus' preparation differs from others in respect to the pyrimidine nucleus present.

It should be noted that the absorption for Peters' material as published by Heyroth and Loofbourow differs somewhat from that of all preparations by other workers and also that the absorption which Peters and Philpot report is radically different from Heyroth and Loofbourow's curve on material furnished them by Peters. In this connection it should be pointed out that Peters' material also differs from that of all other workers in that its melting point is much lower and more variable[4] so that Peters' substance should be regarded as different in some respect from that of others.

We owe our thanks to Mr. F. Rosebury for some of the spectrograms and to Mr. W. Saschek for many of the analyses. We also wish to acknowledge the financial aid of the Carnegie Corporation of New York through the Carnegie Institution of Washington.

Summary

1. Analyses of the vitamin hydrochloride agree best with the formula $C_{12}H_{16}N_4OS$, as calculated for the hypothetical free base.

2. Ultraviolet absorption occurs in two bands at 235 mμ and 267 mμ, respectively.

NEW YORK CITY RECEIVED OCTOBER 29, 1934

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[13] E. C. C. Baly, R. A. Morton and R. W. Riding, *Proc. Roy. Soc.* (London), **A113**, 709 (1927); H. Von Halban and J. Eisenbrand, *ibid.*, **A116**, 153 (1927).

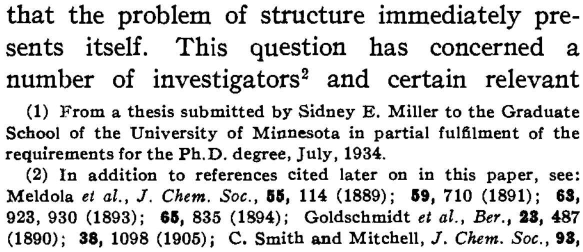
[14] Very recently A. Smakula reports an error in Windaus' absorption results and finds two bands in his material, *Z. physiol. Chem.*, **230**, 231 (1934).

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY OF THE UNIVERSITY OF MINNESOTA]

The Structure of Hydroxyazo Compounds[1]

BY WALTER M. LAUER AND SIDNEY E. MILLER

There are two general methods for the preparation of hydroxyazo compounds: (a) the action of phenylhydrazines on quinones and (b) the coupling of diazonium compounds with phenols.

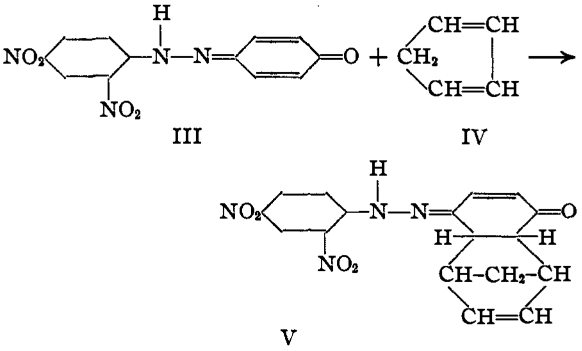


I

II

These two reactions yield the same product, so that the problem of structure immediately presents itself. This question has concerned a number of investigators[2] and certain relevant methods of approach will be introduced in the discussion of the results of the present study.

The fruitful "Diene Synthesis" of Diels and Alder[3] suggested a method of detecting quinoidation, for if the hydroxyazo compounds can exist in a tautomeric quinoid form (I) they should add a 1,4 conjugated diene, since a number of such dienes add to quinones. Accordingly, the addition of cyclopentadiene to various hydroxyazo compounds was investigated. It was found that



III

IV

V

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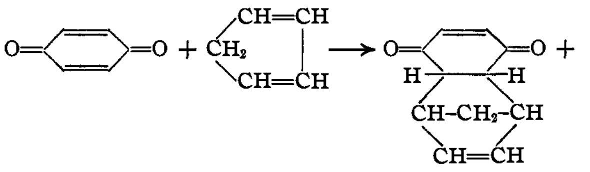
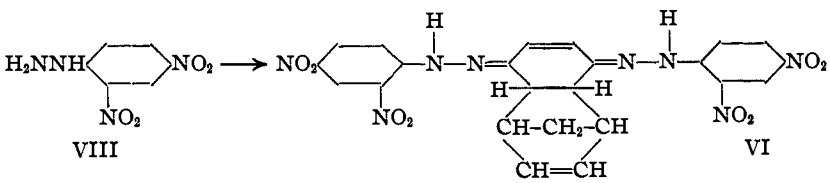
[1] From a thesis submitted by Sidney E. Miller to the Graduate School of the University of Minnesota in partial fulfilment of the requirements for the Ph.D. degree, July, 1934.

[2] In addition to references cited later on in this paper, see: Meldola *et al.*, *J. Chem. Soc.*, **55**, 114 (1889); **59**, 710 (1891); **63**, 923, 930 (1893); **65**, 835 (1894); Goldschmidt *et al.*, *Ber.*, **23**, 487 (1890); **38**, 1098 (1905); C. Smith and Mitchell, *J. Chem. Soc.*, **93**, 843 (1908); v. Auwers *et al.*, *Ber.*, **33**, 1923 (1900); **40**, 2154 (1907); **41**, 403, 420 (1908); **47**, 1297 (1914); *Ann.*, **359**, 336 (1908); **360**, 1, 11 (1908); **365**, 291 (1909); **369**, 209 (1909); **378**, 210 (1910); **487**, 79 (1931); **505**, 283 (1933); Burawoy and Markowitsch, *Ann.*, **503**, 180 (1933); **504**, 71 (1933); Burawoy, *ibid.*, **509**, 60 (1934).

[3] For an excellent review see Abderhalden's "Handbuch der biologischen Arbeitsmethoden," Abt. I, Teil 2, p. 3079–3192, Urban and Schwarzenberg, Berlin, 1933.

2,4-dinitrobenzeneazophenol (III) gave an addition product (V), the composition of which indicated that the azo compound and cyclopentadiene (IV) had combined in equimolar proportions (above). In order to establish the structure of this addition product (V) the following synthesis was attempted; but instead of obtaining V the bis-dinitrophenylhydrazone (VI) resulted.


VII

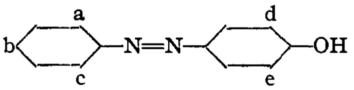

VIII VI

Compound V upon treatment with 2,4-dinitrophenylhydrazine (VIII) gave the same bis-dinitrophenylhydrazone (VI), however, and so structure V was established for the addition product.

Two similar hydroxyazo compounds were found to add cyclopentadiene: 2′,4′-dinitro-4-hydroxy-3-methylazobenzene and 2′,4′-dinitro-4-hydroxy-3-bromoazobenzene. The addition compound from the first of these two azo dyes was converted, as in the case already described, to the bis-dinitrophenylhydrazone and found to be identical with a synthetically prepared sample starting with toluquinone. The hydroxyazo compounds listed in Table I did not undergo addition.

TABLE I

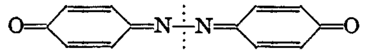
HYDROXYAZO COMPOUNDS WHICH DID NOT FORM ADDITION PRODUCTS WITH CYCLOPENTADIENE

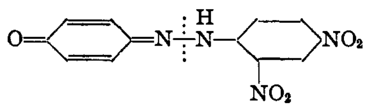


	a	b	c	d	e
(1)	NO_2	NO_2		Br	Br
(2)	NO_2[a]				
(3)	NO_2	Br[d]			
(4)		NO_2[b]			
(5)	NO_2	COOH			
(6)	Br	NO_2	Br		
(7)	COOH[c]				
(8)		SO_3H			
(9)				COOH	
(10)		OH[e]			

[a] Elbs and Keiper, *J. prakt. Chem.*, [2] **67**, 581 (1903). [b] Bamberger, *Ber.*, **28**, 846 (1895). [c] Borsche, *Ann.*, **472**, 216 (1929). [d] Valori, *Atti Acad. Lincei*, [5] **21**, I, 794 (1912). [e] Willstätter and Benz, *Ber.*, **39**, 3492 (1906).

Likewise, the methyl, acetyl and benzoyl derivatives of 2,4-dinitrobenzene azophenol (III) did not yield addition products even though the parent substance is capable of undergoing addition with cyclopentadiene.

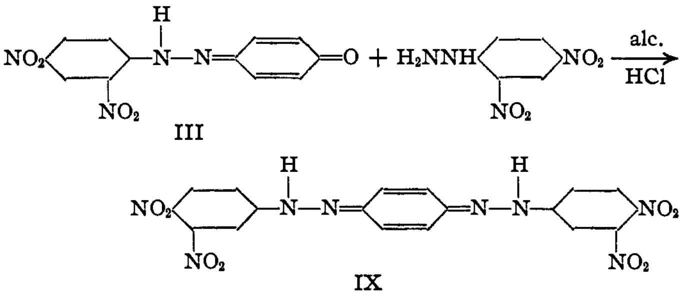

X


III

The addition of two molecules of cyclopentadiene to quinoneazine (X), composed of two parts each of which is identical with the part of III that undergoes addition, confirms the view that addition to III would be expected.

Discussion

Chemical evidence that certain "hydroxyazo" compounds could react as if they were quinones (structure I) was introduced by Borsche[4] and Willstätter.[5] Borsche treated certain hydroxyazo compounds with negatively substituted phenylhydrazines and obtained bis-phenylhydrazones (IX). Thus


III IX

However, this evidence is weakened by the fact that 2′,4′-dinitrobenzeneazophenol (III) when heated alone with alcoholic hydrochloric acid also gave compound IX. Two explanations for the formation of IX present themselves: (a) cleavage of III to give dinitrophenylhydrazine and quinone and subsequent reaction of these cleavage prod-

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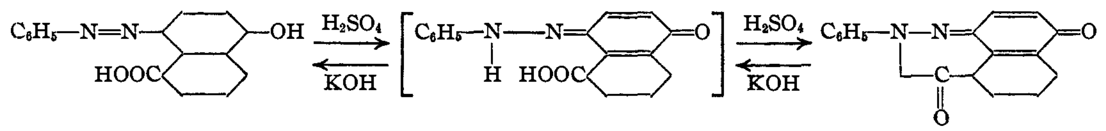
[4] Borsche, Müller and Bodenstein, *Ann.*, **472**, 201 (1929); see also Borsche, *ibid.*, **334**, 143 (1904); **340**, 85 (1905); **343**, 176 (1905); **357**, 171 (1907); **450**, 75 (1926).

[5] Willstätter, Ulbrich, Pogány and Maimeri, *Ann.*, **477**, 161 (1929).

ucts to yield IX; (b) cleavage of III to give dinitrophenylhydrazine and reaction of it with unchanged III. That cleavage did not take place during the process of cyclopentadiene addition is indicated by the following considerations. Had cleavage of III occurred in the presence of cyclopentadiene to give VII, then VI would have been the product instead of V. None of compound VI was ever encountered in the reaction mixture of the addition reaction. Furthermore, III, when subjected to the action of glacial acetic acid under the conditions which were maintained during the formation of the addition product, was recovered unchanged with a 97% yield and, finally, the same addition product (V) was prepared in benzene solution. These experiments therefore show that certain "hydroxyazo" compounds are capable of exhibiting quinoid behavior (structure I).

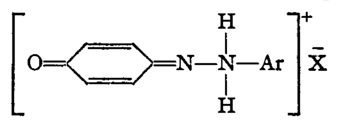
In agreement with the work of Borsche it has been found that only negatively substituted "hydroxyazo" compounds can show this behavior. Inner salt formation or chelation involving a nitro group and the imino group may stabilize the quinoid structure of III. That such an interaction is probable is indicated by some work of Willstätter,[5] which is most briefly described as follows



XI XII XIII

The formation of the lactam (XIII) is best explained by postulating the existence of the intermediate quinone hydrazone (XII).

Addition experiments in which the acidity of the medium was changed are described in the experimental part of the present work. The yields of addition product V in case of (a) one molar sodium acetate in glacial acetic acid (20.1%), (b) glacial acetic acid (29.4%), (c) one molar chloroacetic acid in glacial acetic acid (37.2%) and (d) two molar chloroacetic acid in glacial acetic acid (46.3%) show an increase with increased acidity of the medium. These results are consistent with the view that salt formation of the type



takes place.[6]

The alkyl derivatives of the "hydroxyazo" compounds upon reductive splitting yield ethers of aminophenols and the products obtained by coupling diazonium salts with phenol ethers are identical with those obtained by coupling with phenols and then alkylating. They are O-substitution products, therefore.[7] Acylation leads to similarly constituted derivatives. McPherson synthesized N-acyl substitution products from quinones and *as*-acyl phenylhydrazines. The N-acyl derivatives prepared in this manner were not identical with those obtained by acylating the hydroxyazo compounds.[8] Non-addition of cyclopentadiene to alkyl and acyl derivatives of the hydroxyazo compounds is therefore to be expected since O-substitution renders structure II incapable of tautomerization to structure I.

Experimental

2,4-Dinitrobenzeneazophenol (III) was prepared in two different ways.

(a) **From quinone and 2,4-dinitrophenylhydrazine** according to the method of Borsche.[9]

(b) **By Coupling Diazotized 2,4-Dinitraniline with Phenol.**—Seven and six-tenths grams (0.11 mole) of sodium nitrite was added slowly with vigorous stirring to 40 cc. of concd. sulfuric acid cooled in an ice bath. After all the nitrite had been added, the mixture was warmed to not more than 65° until all of the nitrite dissolved. The solution was then cooled and 18.3 g. (0.1 mole) of the dinitroaniline was added in portions with stirring. After standing at 10–15° for four hours, the diazotized amine was added to about one liter of ice and water and filtered. The clear yellow solution obtained was added slowly with stirring to a solution of 9.4 g. (0.1 mole) of phenol in 300 cc. of glacial acetic acid containing 150 g. of sodium acetate. During the first part of this addition, the temperature was kept at 10–15° to prevent freezing, but after about 100 cc. of the diazonium solution had been added the temperature was lowered to 5°. After all of the diazonium solution was added, the reaction mixture was stirred for one-half hour, filtered and washed thoroughly with cold

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(6) Hewitt *et al.*, *J. Chem. Soc.*, **77**, 99, 712, 810 (1900); **79**, 155, 160 (1901); **81**, 171, 1202 (1902); **89**, 182 (1906).

(7) Meldola and Morgan, *J. Chem. Soc.*, **55**, 603, 604, 608 (1889); Meldola and Hanes, *ibid.*, **65**, 841 (1894); Meldola and Southern, *Proc. Chem. Soc.*, **10**, 118 (1894); Jacobson and Fischer, *Ber.*, **25**, 992, 1013 (1892); Jacobson, *ibid.*, **26**, 681, 688, 700 (1893); **27**, 2700 (1894); *Ann.*, **287**, 97 (1895); Nolting, *Ber.*, **23**, 3257 (1890); Schmidt, *ibid.*, **38**, 3201, 4022 (1905); Charrier and Ferreri, *Gazz. chim. ital.*, **43**, II, 148, 211, 227 (1913); **44**, I, 120, 165, 405 (1914); K. Meyer and Lenhardt, *Ann.*, **398**, 74 (1913); Meyer, Irschick and Schlösser, *Ber.*, **47**, 1741 (1914).

(8) McPherson, *ibid.*, **28**, 2414 (1895); *Am. Chem. J.*, **22**, 362 (1899); Willstätter and Veraguth, *Ber.*, **40**, 1432, 3971 (1907).

(9) Borsche, *Ann.*, **357**, 180 (1907).

water. The weight of air dried product was 23–24 g. (*ca.* 80%). The product crystallized from methyl alcohol melted at 185–186°. A mixture of the azo compound from (a) and (b) showed no melting point depression.

(c) **The acetyl derivative** was prepared by treating 2.2 g. in 10 cc. of pyridine with 1.4 cc. of acetic anhydride. The red product, obtained by pouring the mixture into water, when crystallized from acetic acid, weighed 1.9 g. (76%) and melted at 135–136°.

(d) **The benzoyl derivative** was prepared according to the method of Borsche.[10] The melting point 163.5–164.5° checked his value.

(e) **The methyl ether** was obtained by methylating with dimethyl sulfate in 2 *N* sodium hydroxide. The product crystallized from ethyl acetate melted at 176–177°.

2′,4′-Dinitro-4-hydroxy-3-methylazobenzene has been prepared by Borsche[10] from toluquinone and 2,4-dinitrophenylhydrazine. The present authors have been unable to check the melting point reported by him (127–128°) by using (a) his method of preparation or (b) by preparing the dye by coupling diazotized 2,4-dinitraniline with *o*-cresol. The value found in both cases in this Laboratory was 172.5–173.5°. Crystallization of the product from aqueous alcohol or aqueous acetic acid gave an orange-yellow product melting at 158–160°. In some cases this melting point would persist after several crystallizations from the above solvents. In others, one crystallization was sufficient to raise the melting point to 172.5°. The product when crystallized from benzene formed red needles.

Anal. Calcd. for $C_{13}H_{10}O_5N_4$: C, 51.64; H, 3.34. Found: C, 51.64; H, 3.48.

The benzoyl derivative was prepared from the dye obtained by (a) and (b). Both samples melted at 182–183° and a mixed melting point showed no lowering.

2′,4′-Dinitro-4-hydroxy-3-bromoazobenzene was prepared as follows. One and eighty-seven hundredths grams (0.01 mole) of bromoquinone in 40 cc. of alcohol was treated with a solution of 1.98 g. (0.01 mole) of 2,4-dinitrophenylhydrazine in 175 cc. of alcohol in the presence of 1 cc. of concd. hydrochloric acid. After the reaction mixture had stood for about one hour water was added to cloudiness. Upon cooling, three and eight-tenths grams of the azo compound separated out. Crystallization from methyl alcohol or benzene gave reddish-brown needles melting at 186–187°.

Anal. Calcd. for $C_{12}H_7O_5N_4Br$: C, 39.23; H, 1.92. Found: C, 39.59; H, 2.05.

2′,4′-Dinitro-4-hydroxy-3,5-dibromoazobenzene.—Bromination of 2′,4′-dinitro-4-hydroxyazobenzene and the action of 2,6-dibromoquinone upon 2,4-dinitrophenylhydrazine gave identical products; m. p. 211–212°. The *benzoyl* derivative which melted at 198–199° was analyzed.

Anal. Calcd. for $C_{19}H_{10}O_6N_4Br_2$: C, 41.5; H, 2.14. Found: C, 41.8; H, 1.83.

2′,6′-Dibromo-4′-nitro-4-hydroxyazobenzene.—Sodium nitrite (3.8 g.) was dissolved in 30 cc. of sulfuric acid, cooled in the ice-bath and 14.8 g. of 2,6-dibromo-4-nitraniline added with stirring. After standing, it was poured into 700 cc. of ice and water and filtered. The diazonium solution, added to 4.7 g. of phenol and 30 g. of sodium acetate in 125 cc. of acetic acid and cooled to 5° formed a reddish colored azo compound. After saturating the solution with sodium acetate, the dye was separated by filtration. It was purified by crystallization from aqueous methyl alcohol as reddish-brown needles; m. p. 148–150°. Crystallization from chloroform raised the m. p. to 153–153.5°.

Anal. Calcd. for $C_{12}H_7O_3N_2Br_2$: C, 35.9; H, 1.76. Found: C, 36.5; H, 1.78.

2′-Nitro-4′-carboxy-4-hydroxyazobenzene.—Diazotized 3-nitro-4-aminobenzoic acid coupled with phenol gave this compound in 72% yield. After two crystallizations from aqueous methyl alcohol it melted at 252–254°. *The acetyl derivative* melted at 214–216° and was analyzed.

Anal. Calcd. for $C_{15}H_{11}O_6N_3$: C, 54.69; H, 3.37. Found: C, 54.73; H, 3.46.

Addition Studies

(1) **The Addition of Cyclopentadiene to 2,4-Dinitrobenzeneazophenol.**—Two and one-half grams of 2,4-dinitrobenzeneazophenol in 25 cc. of glacial acetic acid was treated with 5 cc. of freshly distilled cyclopentadiene and refluxed gently on a steam-bath for five hours. The flask containing the reaction mixture was then well stoppered and placed in an ice box for ten days. During this period 0.5 g. of dark red crystals, green by reflected light, formed. The crude material melted at 161–163°, but when crystallized from acetic acid, ethyl acetate, pyridine or acetic anhydride the melting point was raised to 167–168°. The addition product was insoluble in aqueous alkali.

The same addition product in 17.6% yield was obtained in a somewhat similar manner using benzene as solvent. The melting point was low, 160–161°, but crystallization raised this to 166–167°.

Anal. Calcd. for $C_{17}H_{14}O_5N_4$: C, 57.63; H, 3.98; N, 15.82. Found: C, 57.91; H, 3.99; N, 16.13.

A hydrazone was formed with 2,4-dinitrophenylhydrazine and it was by means of this bis-dinitrophenylhydrazone that the structure of the addition compound was established.

(2) **Cyclopentadiene-*p*-benzoquinone-bis-(2,4-dinitrophenylhydrazone).** (a) **From 2,4-Dinitrophenylhydrazine and the Addition Product of Cyclopentadiene and 2,4-Dinitrobenzeneazophenol.**—The addition compound (0.3 g.) described in (1) was suspended in 10 cc. of absolute ethyl alcohol and 0.4 g. of 2,4-dinitrophenylhydrazine dissolved in 30 cc. of alcohol containing three drops of concentrated hydrochloric acid was added and the mixture refluxed two to three hours on a steam cone. During the heating the addition compound dissolved slowly and a darker red substance precipitated out. After filtration, it was crystallized by dissolving in nitrobenzene, filtering, adding alcohol until cloudy and then cooling. The product, dark red crystals (bluish by reflected light), melted at 258–260° with decomposition. The yield of recrystallized material was 64%.

Anal. Calcd. for $C_{23}H_{18}O_8N_8$: N, 20.97. Found: N, 20.86.

(b) **From Cyclopentadiene-*p*-benzoquinone and 2,4-Dinitrophenylhydrazine.**—Eighty-two hundredths gram of

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[10] Borsche, *Ann.*, **472**, 212 (1929).

the cyclopentadiene-*p*-benzoquinone addition compound was dissolved in 15 cc. of absolute ethyl alcohol and cooled in an ice-bath. To this solution, 1.98 g. of 2,4-dinitrophenylhydrazine dissolved in 200 cc. of alcohol containing 2 cc. of concd. hydrochloric acid, was added, and the reaction mixture placed in an ice box overnight. Two and one-half grams (90% yield) of a red reaction product formed. After purification as in (a) it melted at 259-260° with decomposition. A mixed melting point with the product in (2a) showed no lowering.

Anal. Calcd. for $C_{23}H_{18}O_8N_8$: C, 51.67; H, 3.39; N, 20.97. Found: C, 51.91; H, 3.38; N, 21.45.

A microscopic examination of the two crystalline products, (a) and (b), was made by Dr. E. B. Sandell of the Division of Analytical Chemistry of the University of Minnesota and we are indebted to him for the following report: "Both preparations consist of orange crystals, prismatic in habit. The optical properties of the two are the same in the following respects: strong double refraction, small extinction angles, negative elongation and marked pleochroism (yellow-orange to orange-red). The refractive indices of the prism views of both samples are greater than 1.74. No differences in properties were observed. The two preparations appear to be identical."

(3) **The Influence of Acidity on Additions in Glacial Acetic Acid.**—In this series of studies the addition of cyclopentadiene to 2,4-dinitrobenzeneazophenol was carried out in glacial acetic acid, one molar sodium acetate in glacial acetic acid, and one and two molar chloroacetic acid in glacial acetic acid. One determination was also made in a one molar solution of trichloroacetic acid in glacial acetic acid but in this case the crystalline product was contaminated with tarry material; the azo compound appears to be slightly unstable in this solvent.

The procedure in every case was the same; 1.440 g. of the azo compound was dissolved in 20 cc. of the solvent and 3 cc. of freshly distilled cyclopentadiene was added. The reaction mixture was refluxed at 58–60° for three hours, cooled, stoppered and set in an ice-box at 10–15° for ten days.

The analysis of the reaction mixture was carried out as follows. After filtration the crystalline addition product was washed with 25 cc. of cold glacial acetic acid. The material obtained in this manner (melting 3 to 4° low) was weighed as addition product (A). The filtrate and washings were added to 300 cc. of water containing 25–30 g. of sodium acetate and the precipitate, which consisted of a mixture of the azo dye and the addition compound, was washed with water, dried in a desiccator and weighed (C). The dried mixture was then treated with 25 cc. of boiling methyl alcohol, cooled, filtered and the addition compound obtained in this way was washed with 25 cc. of cold methyl alcohol. After drying, it was weighed (B). This weight (B) added to the weight (A) of addition product previously obtained gives the total amount of addition product, while (C) minus (B) gives the amount of uncombined azo dye (see Table II).

(4) **The Action of Cyclopentadiene upon the Acetyl, Benzoyl and Methyl Derivatives of 2,4-Dinitrobenzeneazophenol.**—Cyclopentadiene failed to give an addition compound; the acetyl, benzoyl and methyl derivatives were recovered in approximately 90% yields and mixed melting points with starting materials showed no lowering.

TABLE II

ADDITION OF CYCLOPENTADIENE TO 2,4-DINITROBENZENEAZOPHENOL, 1.440 GRAMS

Solvent	Yield of addition cpd. G.	%	Azo cpd. recovered G.	%	Total material accounted for %
1 *M* CH_3COONa	0.358	20.2	1.138	79.0	99.2
	.355	20.1	1.153	80.0	100.1
CH_3COOH	.490	27.7	1.042	72.4	100.1
	.540	30.5	1.039	72.1	102.6
	.550	31.1	0.987	68.5	99.6
	.503	28.4	...	..	..
1 *M* $ClCH_2COOH$	.653	36.9	0.954	66.2	103.1
	.656	37.1	1.007	69.9	107.0
	.667	37.7	...	..	...
2 *M* $ClCH_2COOH$	.836	47.2	0.739	51.3	98.5
	.860	48.6	.742	51.5	100.1
	.803	45.4	.757	52.5	97.9
	.781	44.1	...	..	...

(5) **The Addition of Cyclopentadiene to 2′,4′-Dinitro-4-hydroxy-3-methylazobenzene.**—One and fifty-one hundredths grams of the azo compound was dissolved in 20 cc. of glacial acetic acid, and 3 cc. of freshly distilled cyclopentadiene was added as in the case of 2,4-dinitrobenzeneazophenol. The red crystalline addition product (1.01 g.), after several crystallizations from a mixture of benzene and methyl alcohol, melted at 162–163°. It is insoluble in cold aqueous alkalies, but dissolves in alcoholic sodium methoxide to give a deep blue-violet color.

Anal. Calcd. for $C_{18}H_{16}O_5N_4$: C, 58.67; H, 4.38. Found: C, 58.74; H, 4.28.

(6) **The Preparation of Cyclopentadiene-toluquinone-bis-2,4-dinitrophenylhydrazone. (a) From Addition Product (See Section 5) and 2,4-Dinitrophenylhydrazine.**—Thirty-seven hundredths gram of addition product in 50 cc. of warm alcohol was treated with 0.2 g. of dinitrophenylhydrazine in 30 cc. of alcohol containing 2 drops of concd. hydrochloric acid and refluxed for two to three hours on a steam-bath and then placed in an ice box overnight. A bluish-black crystalline product formed which melted at 252–253° with decomposition; yield 0.26 g. (47%).

Anal. Calcd. for $C_{24}H_{20}O_8N_8$: C, 52.5; H, 3.68. Found: C, 52.7; H, 3.84.

(b) **From Cyclopentadiene-toluquinone and 2,4-Dinitrophenylhydrazine.**—Forty-seven hundredths gram of the addition product of cyclopentadiene to toluquinone, dissolved in 20 cc. of methanol, was added slowly to one gram of 2,4-dinitrophenylhydrazine in 125 cc. of methanol containing 0.6 cc. of concd. hydrochloric acid. After refluxing for several hours, the reaction mixture was placed in an ice box overnight. A maroon colored product (0.55 g.) resulted. Crystallization from nitrobenzene or pyridine yielded a product which melted at 252–253° and a mixed melting point with the product from (a) showed no lowering.

Anal. Calcd. for $C_{24}H_{20}O_8N_8$: C, 52.5; H, 3.68. Found: C, 52.7; H, 3.69.

(7) **The Addition of Cyclopentadiene to 2′,4′-Dinitro-4-hydroxy-2-bromoazobenzene.**—A mixture of 0.8 g. of the

dye, 20 cc. of glacial acetic acid and 2 cc. of cyclopentadiene yielded a blue-black crystalline addition compound (0.16 g.). After two crystallizations from acetic acid, it melted at 176–177°.

Anal. Calcd. for $C_{17}H_{13}O_5N_4Br$: C, 47.11; H, 3.03. Found: C, 47.36; H, 3.13.

(8) **The Addition of Cyclopentadiene to Quinoneazine.**—One and one-tenth grams of quinoneazine dissolved in 15 cc. of benzene and cooled in an ice-bath was treated with 2 cc. of cyclopentadiene. At the end of two days, a small amount of the unreacted azine was filtered off and the filtrate evaporated to dryness under reduced pressure to give 0.7 g. of product. After twice dissolving in ethyl acetate and precipitating with petroleum ether, it was crystallized from ethyl acetate alone. Four crystallizations gave a yellow crystalline product melting at 149–150°. Analysis showed the addition of two molecules of cyclopentadiene.

Anal. Calcd. for $C_{22}H_{20}O_2N$: C, 76.69; H, 5.86. Found: C, 76.36; H, 6.12.

Summary

Cyclopentadiene adds to certain hydroxyazo compounds; a result which provides definite evidence that these substances may show quinoid character. The extent of addition is influenced by the acidity of the medium and its occurrence is not a general property of the *p*-hydroxyazo compounds.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF STANFORD UNIVERSITY]

Saponins and Sapogenins. III. The Sapogenins Obtained from Chlorogalum Pomeridianum

BY POE LIANG[1] AND C. R. NOLLER

Of the various plants used as fish poisons by the California Indians, the most important was a member of the lily family, *Chlorogalum pomeridianum* Kunth,[2] which is commonly known as California soap plant, or *amole*, the latter term being one commonly assigned by the Spanish-Californians and Mexicans to any of the various plants used as soap substitutes.

The only chemical investigation of this plant recorded in the literature is that of Trimble,[3] who reported a saponin content of 6.95% of the dry bulb. Our work shows that hydrolysis of the crude alcoholic extract of the bulbs yields two sapogenins which are especially interesting as they contain twenty-six carbon atoms and hence are related to the sapogenins obtained by the hydrolysis of the digitalis saponins, and by the hydrolysis of parillin, one of the sarsaparilla saponins,[4] instead of being triterpenoids like most sapogenins that have been carefully investigated.[5] One sapogenin has the empirical formula $C_{26}H_{42}O_4$,[6] and hence is isomeric with gitogenin which is obtained from gitonin, one of the digitalis saponins.[7] Like gitogenin it has two hydroxyl groups and two oxygen atoms which do not give reactions for any reactive type of oxygen linkage and hence must be assumed for the present to be of the ethylene oxide type. No color is obtained with tetranitromethane, indicating the absence of a double bond. The melting point of this sapogenin is 273–276° compared with 271–272° for gitogenin.[7] The compounds are not identical, however, because a marked depression was observed on taking a mixed melting point.[8] Moreover, the dibenzoate of the new sapogenin melts at 200–204° whereas gitogenin dibenzoate melts at 223–225°.[9] Since this sapogenin appears to be different from any previously reported, we have named it *chlorogenin*.

The other sapogenin isolated has the empirical formula $C_{26}H_{42}O_3$ and is isomeric with sarsasapogenin obtained from several varieties of smilax[10]

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[1] Research Fellow of the China Foundation for the Promotion of Education and Culture, 1933–34.

[2] Chestnut, *Contributions from the U. S. National Herbarium*, **7**, 319 (1902).

[3] Trimble, *Am. J. Pharm.*, **62**, 598 (1890).

[4] Jacobs and Simpson, *J. Biol. Chem.*, **105**, 501 (1934).

[5] Ruzicka and co-workers, *Helv. Chim. Acta*, **15**, 431 (1932).

[6] The difficulty of obtaining satisfactory combustion data on the sapogenins is well known. Our own analyses have all been by macrocombustion and the results for carbon do not agree well with the composition assigned to these sapogenins. In one instance we have had the sapogenin analyzed by a commercial microanalyst[15] and by a microanalyst[16] accustomed to the analysis of sapogenins. We consider it significant that the results of the latter[16] are in good agreement with the proposed formula. Of our own analyses we lay most stress on the saponification of the benzoates, which gave results readily reproducible with high accuracy and which support the formulas proposed.

[7] Windaus and Schneckenburger, *Ber.*, **46**, 2628 (1913).

[8] We are greatly indebted to Dr. W. A. Jacobs of the Rockefeller Institute for Medical Research for supplying us with pure samples of gitogenin, tigogenin and sarsasapogenin.

[9] Windaus and Brunken, *Z. physiol. Chem.*, **145**, 37 (1925).

[10] Van der Haar, *Rec. trav. chim.*, **48**, 726 (1929). It is of interest that *Smilax*, like *Chlorogalum*, is a member of the lily family.