three times, it melted at 220–225°, $[\alpha]^{27.3}D$ +73 ± 3.5° (*c* 0.2, chloroform, *l* 4). For analysis it was dried *in vacuo* at 130°.

Anal. Calcd. for $C_{36}H_{54}O_8$: C, 70.3; H, 8.85; CH_3CO, 14.00; CH_3O, 10.10; mol. wt., 614.8. Found: C, 69.9; H, 8.73; CH_3CO,[17] 14.0; CH_3O, 10.1; mol. wt. (Rast), 620.

Sapogenin Bromolactone.—Sapogenin obtained directly from saponin hydrolyzate was brominated in methanol–carbon tetrachloride solution as described by Winterstein and Egli[19] for the preparation of a bromolactone of siaresinolic acid. The product was dissolved in ether and dried over anhydrous sodium sulfate. Attempts to crystallize it from the usual solvents were not successful. It was obtained in form of a white powder by adding water to the methanol solution, and filtering.

Anal. Calcd. for $C_{30}H_{45}O_6Br$: Br, 13.74; mol. wt., 581.6. Found: Br, 14.3; equiv. wt., 570 (titration of remaining carboxyl).

In acetic acid solution the product gave no color with tetranitromethane.

Acknowledgments.—We wish to acknowledge the assistance of K. J. Palmer, D. R. Black and Francis T. Jones for crystallographic data on the sapogenin diacetate, L. M. White for elemental analyses, and A. Bevenue for identification of sugars from the sapogenins. We also acknowledge the technical assistance of J. Guggolz and I. V. Ford.

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(19) Alfred Winterstein and Robert Egli, *Z. physiol. Chem.*, **202**, 207 (1931).

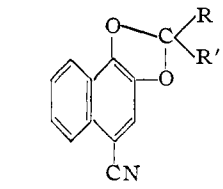
(20) Agricultural Research Service, U. S. Department of Agriculture. Article is not copyrighted.

Action of Diazoalkanes on *o*-Quinones and Other Carbonyl (Thiocarbonyl) Compounds with Special Reference to the Nature of the Intermediate Compounds

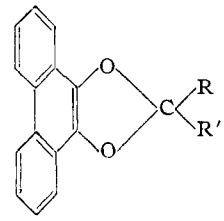
BY ALEXANDER SCHÖNBERG, AHMED MUSTAFA, WILLIAM IBRAHIM AWAD AND (in part) GAMAL EL-DIN MOHAMED MOUSSA

RECEIVED JULY 15, 1953

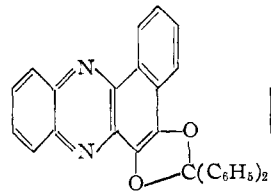
Formation of methylene ethers (such as I, II and III) by the reaction of *o*-quinones with diazoalkanes seems to be general.[1] In one case, however, the reaction of phenanthraquinone with diazomethane, both the ether IIa[2] and the ethylene oxide V[3] are obtained. We have examined the reactions of phenanthraquinone, 4-cyano-β-naphthoquinone and 1,2-benzophenazine-3,4-quinone with a few diazoalkanes, and in all cases obtained a single product (Ia-Ic, IIb, III, see Table I). The substances are assigned the ether structures as shown, not only on analogy with similar products, but also because they show greater stability to acid hydrolysis than would be expected if they contained an ethylene oxide ring. Under more drastic acid hydrolysis, however, they are cleaved to the corresponding dihydroxy compounds. Thus we obtained 1,2-dihydroxy-4-cyanonaphthalene from Ic; the product from hydrolysis of III is IV, a tautomeric form of the expected 3,4-dihydroxy-1,2-benzophenazine.[4] Similar hydrolysis of the diphenylmethylene ether of 9,10-dihydroxyphenanthrene has been noted.[5]



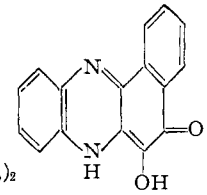
Ia, R = H; R′ = CH_3
Ib, R = R′ = 9-fluorenylidene ($C_{13}H_8$)
Ic, R = R′ = C_6H_5



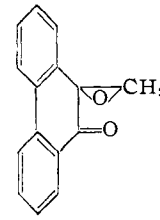
IIa, R = R′ = H
IIb, R = CH_3, R′ = H
IIc, R = R′ = C_6H_5



III



IV



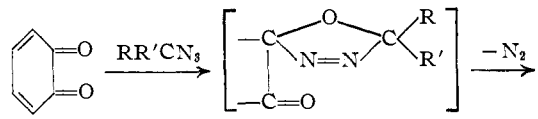
V

TABLE I

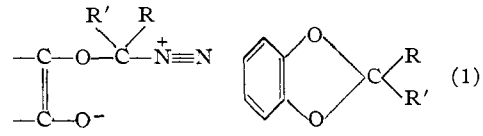
LIST OF THE NEW DERIVATIVES OF METHYLENE ETHERS

4-Cyano-1,2-(methylmethylenedioxy)-naphthalene (Ia)
4-Cyano-1,2-(diphenylmethylenedioxy)-naphthalene (Ic)
(4-Cyano-1,2-naphthylenedioxy)-9-fluorene (Ib)
9,10-(methylmethylenedioxy)-phenanthrene (IIb)
3,4-(Diphenylmethylenedioxy)-1,2-benzophenazine (III)

We consider the reaction of diazoalkanes with *o*-quinones follows a course similar to that of the diazoalkanes with olefins and can be formulated as shown in (1); the nitrogen-containing intermediate A can also be written as B. An analogous reaction occurs in the case of the reaction of diazoalkanes with *o*-quinoneimines and *o*-quinone monoximes.[6]



A



B (1)

A slightly different mechanism originally proposed by Arndt is generally accepted[7] for the reaction of diazoalkanes with monoketones and aldehydes.

(1) L. Fieser and J. L. Hartwell, THIS JOURNAL, **57**, 1479 (1935); A. Schönberg and A. Mustafa, *J. Chem. Soc.*, 746 (1946); A. Schönberg, W. I. Awad and N. Latif, *ibid.*, 1368 (1951); L. Horner and E. Lingnau, *Ann.*, **573**, 30 (1951); A. Schönberg and N. Latif, *J. Chem. Soc.*, 446 (1952); A. Schönberg, A. Mustafa and S. M. D. Zayed, THIS JOURNAL, **75**, 3402 (1953).

(2) H. Biltz and H. Paetzold, *Ann.*, **433**, 71, 83 (1923).

(3) F. Arndt, J. Amende and W. Ender, *Monatsh.*, **59**, 210 (1932).

(4) G. Badger, R. S. Pearce and R. Pettit, *J. Chem. Soc.*, 3204 (1951).

(5) A. Schönberg and A. Mustafa, *ibid.*, 746 (1946).

(6) A. Schönberg and W. I. Awad, *ibid.*, 72 (1950).

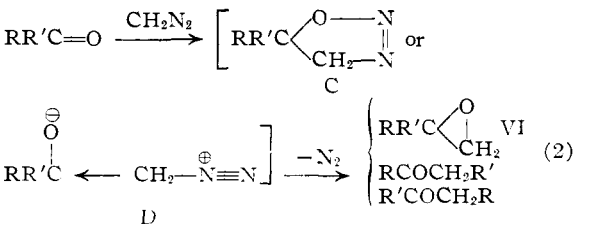
(7) *Cf. inter alia* (a) E. E. Turner and M. M. Harris, "Organic Chemistry," Longmans and Co., London, 1952, p. 148; (b) B. Eistert in "Newer Methods of Preparative Organic Chemistry," Interscience Publishers, Inc., New York, N. Y., 1948, p. 522; see N. V. Sidgwick, "The Organic Chemistry of Nitrogen," 1942, p. 359.

TABLE II

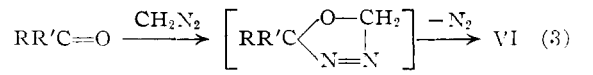
NEW METHYLENE ETHERS OF 1,2-QUINONES

1,2-Quinone	Reactants: Diazoalkane	Reaction product (yield in g.)	M.p., °C.	Time of reaction and temp.	Solvent for cryst.[e]	Formula	Analyses, %	Color with H_2SO_4
Phenanthraquinone (1.5 g.)	Diazoethane[a] (from nitrosoethylurea) (40 ml. ether)	IIb[c]	65	24 hr. at 0°	A	$C_{16}H_{12}O_2$	Calcd.: C, 81.3; H, 5.0 Found: C, 81.4; H, 5.3	
4-Cyano-1,2-naphthoquinone (0.85 g.)	Diazoethane (from 6.2 g. nitrosoethylurea)	Ia[c] (0.3)	108–109	2 hr. at 0°	A	$C_{13}H_9O_2N$	Calcd.: C, 73.9; H, 4.3; N, 6.6 Found: C, 74.0; H, 4.1; N, 6.7	Red-brown
4-Cyano-1,2-naphthoquinone (1.8 g.)	Diphenyldiazomethane[b] from 2.6 g. benzophenonehydrazone, (30 ml. of benzene)	Ic[c] (2.1)	168	2 hr. at 0°	A	$C_{24}H_{15}O_2N$	Calcd.: C, 82.5; H, 4.3; N, 4.0 Found: C, 82.5; H, 4.4; N, 3.2	Red
4-Cyano-1,2-naphthoquinone (0.5 g.)	9-Diazofluorene (0.95 g.) (30 ml. of benzene)	IIb[d] (0.7)	216–218	Few min. on steam-bath and 48 hr. at room temp.	B	$C_{24}H_{13}O_2N$	Calcd.: C, 83.0; H, 3.8; N, 4.0 Found: C, 83.0; H, 3.8; N, 3.9	Brown olive-green
1,2-Benzophenazine-3,4-quinone (0.5 g.)	Diphenyldiazomethane from 3 g. of benzophenonehydrazone, (60 ml. of benzene)	III	238	Refluxed 2.5 hr. in benzene	C	$C_{29}H_{18}O_2N_2$	Calcd.: C, 81.7; H, 4.2; N, 6.6 Found: C, 81.7; H, 4.2; N, 6.4	Green

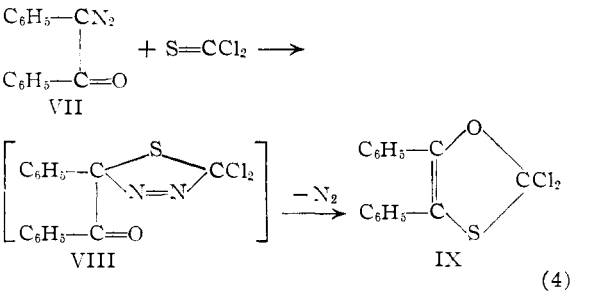
[a] E. A. Werner, *J. Chem. Soc.*, 115, 1096 (1919). [b] H. Staudinger and A. Gaule, *Ber.*, 49, 1897 (1916). [c] Colorless crystals. [d] Light-yellow crystals. [e] A, methyl alcohol; B, dilute acetone, and C, xylene.



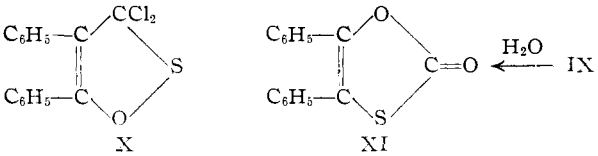
We consider that the reaction between diazoalkanes and monoketones or aldehydes could also proceed according to that outlined by scheme 3 similar to that postulated above for the reaction of *o*-quinones (*cf.* 1). In fact the reaction may well involve both reaction paths, the actual course taken depending on catalytic influences, the more or less electrophilic nature of the groups R and R′ and steric factors. By contrast a nitrogen-containing intermediate similar to C or D cannot be advanced to explain the formation of methylene ethers from *o*-quinones (see I–III).



Action of Phenylbenzoyldiazomethane (VII) with Thiophosgene (VIII).—We suggest that this action follows a course similar to that of diazoalkanes with *o*-quinones leading to IX. This formulation was rejected by Staudinger[8] because its product of alkaline hydrolysis is desoxybenzoin rather than thiobenzoin ($C_6H_5COCH(SH)C_6H_5$). However, alkali converts thiobenzoin into desoxybenzoin.[9] 2,2-Dichloro-4,5-diphenyl-1,3-thioxole (IX) rather than X advanced by Staudinger explains the ready re-



placement of the chlorine atoms by oxygen effected by moisture of the air; we regard the product as 2-oxo-4,5-diphenylthioxole (XI). A reaction related to scheme 4 is that of thiourea with benzoyldiazomethane.[10]



Experimental[11]

Action of Diazoalkanes on Phenanthraquinone, 4-Cyano-1,2-naphthoquinone and 1,2-Benzophenazine-3,4-quinone.

(8) H. Staudinger and J. Siegwart, *Helv. Chim. Acta*, **3**, 845 (1920).

(9) A. Schönberg and Y. Iskander, *J. Chem. Soc.*, 92 (1942).

(10) L. C. King and F. M. Miller, THIS JOURNAL, **71**, 367 (1949); ref. 6.

(11) The experiments with phenanthraquinone and with 1,2-benzophenazine-3,4-quinone were carried out with N. Latif and S. M. D. Zayed, respectively.

—The reaction was carried out by adding gradually the 1,2-quinone to an ethereal solution of diazoethane or 9-diazofluorene or to the benzene solution of diphenyldiazomethane (*cf.* Table II). The final reaction products were obtained by evaporation of the solvent and working up the residues as follows.

The crystals that separated after evaporation of the solvent in the case of IIb, were washed with methyl alcohol.

The oil residue obtained in the case of Ia was triturated with hot methyl alcohol and kept aside in the ice-chest overnight and the brownish solid that separated was then crystallized.

The solid residue obtained in the case of Ic was treated with few ml. of ether, filtered and crystallized.

The yellowish-brown solid deposit in the case of Ib, that was obtained on cooling the reaction mixture, was crystallized.

The solid residue obtained in the case of III was washed with cold benzene and crystallized.

Action of Hydrochloric Acid. (a) IIb.—A solution of 0.2 g. of IIb in a mixture of 30 ml. of methyl alcohol and 5 ml. of concentrated hydrochloric acid (d. 1.18) was refluxed for 40 minutes. The reaction mixture was concentrated and the cooled solution was then poured into ice and the crystals that separated after some time were collected and shown to be unchanged IIb (m.p. and mixed m.p.). The yield was almost quantitative.

(b) Ic.—A suspension of 0.1 g. of Ic and 12 ml. of concentrated hydrochloric acid was heated (steam-bath) for 20 minutes. The mixture was cooled, filtered off and the solid was crystallized from dilute methyl alcohol in colorless crystals. Ic was recovered almost quantitatively (m.p. 168° and mixed m.p.).

The above experiment was repeated, using 0.25 g. of Ic and 20 ml. of concentrated hydrochloric acid and the reaction mixture was refluxed for four hours. It was filtered while hot and left to cool. The colorless crystals, that separated, were recrystallized from dilute alcohol and identified as 1,2-dihydroxy-4-cyanonaphthalene.[12]

(c) III.—The orange solution of 0.2 g. of III in 50 ml. of glacial acetic acid was treated with 1 ml. of concentrated hydrochloric acid. The reaction mixture was heated (steam-bath) for 15 minutes, during which it acquired a deep violet color. The cold reaction mixture was then poured into an ice-water mixture and the blue-violet solid, that separated, was filtered off, washed with alcohol and dried. It was identified as IV by its properties and its transformation into the diacetate of 3,4-dihydroxy-1,2-benzophenazine.[4]

(12) W. Bradley and R. Robinson, *J. Chem. Soc.*, 1484 (1934). We found that 1,2-dihydroxy-4-cyanonaphthalene gives in alcoholic solution a red color with a 15% aqueous titanium trichloride solution (free from iron) (obtained from E. Merck, Darmstadt, Germany).

DEPARTMENT OF CHEMISTRY
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COMMUNICATIONS TO THE EDITOR

THE ENZYMATIC FORMATION OF 4-AMINO-5-IMIDAZOLECARBOXAMIDE RIBOTIDE FROM INOSINIC ACID[1]

Sir:

An enzyme system from pigeon liver, inosinic acid transformylase, has been described which incorporates radioactive formate into the 2-position of inosinic acid.[2] A stimulatory effect of leucovorin on this reaction was demonstrated. The postulation was made that the purine ring was cleaved at the 2-position to form 4-amino-5-imidazolecarboxamide ribotide and that this latter compound reacted with radioactive formate to form inosinic acid-2-C^{14}. It was believed that the citrovorum factor acted as a transformylating coenzyme in these reactions.

Evidence is now presented in further support of this hypothesis. Upon precipitation of pigeon liver extract between 15 and 30% ethanol[3] an enzyme system has been isolated which catalyzes the formation of an aryl amine from inosinic acid in the presence of glycine. This aryl amine compound has been absorbed on Norite, eluted with an ethanol–ammonia solution and placed on a Dowex-1-chloride column at *p*H 9. Upon elution of the column with 0.003 *N* HCl a fraction was obtained which gave a positive test for aryl amine (Bratton–Marshall diazo-reaction).[4,5] The identity of this compound as 4-amino-5-imidazolecarboxamide ribotide is indicated by the demonstration that it contains aryl amine:pentose:organic phosphate in the approximate ratio of 1:1:1. The compound shows an ultraviolet absorption peak at 267 mμ, similar to that of 4-amino-5-imidazolecarboxamide.[6]

A study has been made of the factors involved in the accumulation of the ribotide by the enzyme system. It was found that the reaction rate was highly dependent on the concentration of inosinic acid. Saturation of the enzyme system could not be obtained at a substrate concentration of 20 μM./ml. Glycine has also been shown to be an important factor of the reaction system. Increasing concentrations up to 100 μM./ml. resulted in increased amounts of 4-amino-5-imidazolecarboxamide ribotide formation. Neither L-alanine, L-serine, sarcosine, L-methionine, L-phenylalanine, nor L-leucine can substitute for glycine.

The addition of leucovorin[7] results in a two-to-three fold increase in aryl amine formation. Addition of the natural citrovorum factor,[8] at an equimolar level, resulted in the same stimulation. Addition of a boiled juice of pigeon liver results in a further doubling of the aryl amine formation. These results are shown in the table.

(1) Supported by grants from National Cancer Institute, National Institutes of Health, United States Public Health Service, and the Damon Runyon Memorial Fund for Cancer Research, Inc.

(2) J. M. Buchanan and M. P. Schulman, *J. Biol. Chem.*, **202**, 241 (1953).

(3) W. J. Williams and J. M. Buchanan, *ibid.*, **203**, 583 (1953).

(4) A. C. Bratton and E. K. Marshall, Jr., *ibid.*, **128**, 537 (1939).

(5) J. M. Ravel, R. E. Eakin and W. Shive, *ibid.*, **162**, 463 (1946).

(6) M. R. Stetten and C. L. Fox, Jr., *ibid.*, **161**, 333 (1945).

(7) Kindly supplied by Drs. H. P. Broquist and T. H. Jukes.

(8) Kindly supplied by Dr. J. C. Keresztesy.