yellow. Both bands migrate down the column and eventually into the receiver. The first 200–300 ml. of the solution coming through the bottom of the column is clear and contains very little of the carbohydrazide. The next 400 ml. is yellowish-orange having a *p*H of 4–7 and contains the sodium salt of 1,5-bis-(1-phenyl-4-sulfonic acid) carbohydrazide. Finally, a red solution which contains small amounts of the alkaline form of the carbohydrazide and excess sodium hydroxide comes through the column. This red solution may be discarded. The 400 ml. of yellowish-orange solution is concentrated under reduced pressure at 40–50° with nitrogen being slowly bubbled through to minimize oxidation. The product is precipitated with 95% ethanol, filtered, redissolved in a small volume of water, reprecipitated with alcohol, vacuum dried at room temperature and stored in a desiccator over a solution of calcium chloride at 30% relative humidity. A sample was dried to constant weight at 105–110° and analyzed for sodium by the uranyl zinc acetate method.

Anal. Calcd. for $(NaSO_3 \cdot C_6H_4 \cdot NH \cdot NH)_2C{=}O$: Na, 10.33. Found: Na, 10.13. Loss in weight at 105–110°, 10.86%; loss corresponding to 3 moles of water.

1,5-Bis-(phenyl-4-sulfonic acid) carbohydrazide was also isolated as the barium salt by using 0.13 *M* barium hydroxide for eluting the sulfonic acid from the column. Analysis of this product for barium by the gravimetric barium sulfate method and for water of hydration by drying to constant weight gave results as follows.

Anal. Calcd. for $Ba(SO_3 \cdot C_6H_4 \cdot NH \cdot NH)_2C{=}O$: Ba, 25.54. Found: Ba, 25.47, 25.43. The loss in weight at 105–110° was 8.73%, corresponding to 2.9 moles of water for the above formula.

Yields of the purified material were 10–20% but no attempt was made to establish the maximum yield.

Acknowledgment.—We desire to express our thanks for funds supplied by the John Lee Pratt Trace Analysis Laboratory in partial support of this investigation.

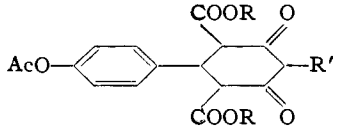
COBB CHEMICAL LABORATORY
UNIVERSITY OF VIRGINIA
CHARLOTTESVILLE, VA. RECEIVED JULY 13, 1951

Synthesis of 1-(*p*-Acetoxyphenyl)-2,6-dicarbethoxy-4-methylcyclohexanedione-3,5. III

BY PHILIPPOS E. PAPADAKIS AND JOSEPH SCIGLIANO

In a previous communication[1] the synthesis of 1-(*p*-acetoxyphenyl)-2,6-dicarbethoxycyclohexanedione-3,5 (I) was reported. The object of the work presented here was the synthesis of a monomethyl derivative at carbon-4 of the cyclohexanedione ring (II). Since the direct methylation of I may result in isomeric monomethyl derivatives the following method was adopted which is assumed to give the desired product.

The sequence of the syntheses involved in this work is: *p*-acetoxybenzaldehyde → ethyl 4-acetoxybenzalmalonate → 1(*p*-acetoxyphenyl)-2,6-dicarbethoxy-4-methylcyclohexanedione-3,5 (II). The synthesis of II was accomplished by condensing and cyclizing the sodio derivative of ethyl β-oxopentanoate with ethyl 4-acetoxybenzalmalonate. Substance (II) will serve not only as reference compound in experiments involving direct methylation of (I) but also as an intermediate in the further synthesis of substances related to steroids and other physiologically important compounds.



I, R′ = H
II, R′ = CH_3

(1) Papadakis, THIS JOURNAL, **67**, 1799 (1945).

Experimental

Reagents.—Ethyl β-oxopentanoate[2] was prepared by Grignard reaction of ethylmagnesium iodide on ethyl cyanoacetate. Ethyl 4-acetoxy-benzalmalonate[3] was prepared from *p*-acetoxybenzaldehyde and diethyl malonate using diethylamine as condensing agent.

1-(*p*-Acetoxyphenyl)-2,6-dicarbethoxy-4-methylcyclohexanedione-3,5 (II).—Ethyl 4-acetoxybenzalmalonate (5.88 g.) was treated with a mixture of equivalent quantities of ethyl β-oxopentanoate (2.8 g.) and CH_3ONa in absolute methyl alcohol. The mixture was refluxed on a water-bath for five hours, the alcohol evaporated and the residue dried for two hours under vacuum (oil-bath temperature 130°).

Upon cooling the solution was acidified with cold 3 *N* HCl, and then 5 cc. in excess. The precipitate was filtered off, washed several times with distilled water and dried in a vacuum desiccator. The dry crystals were washed with ether several times, m.p. 157°. When the crystals are dissolved with absolute ethyl alcohol, the solution gives a yellow ferric chloride test. (The material which dissolved in ether gives a red purple color with $FeCl_3$.)

Anal. Calcd. for $C_{21}H_{24}O_8$: C, 62.37; H, 5.98. Found: C, 62.80; H, 6.19.

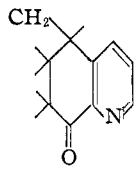
(2) Blaise, *Compt. rend.*, **132**, 970 (1901).
(3) Knoevenagel and Albert, *Ber.*, **37**, 4481 (1904).

CREIGHTON UNIVERSITY
OMAHA, NEBRASKA RECEIVED MAY 31, 1951

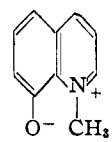
The Reaction of Diazomethane with 8-Quinolinols

BY J. P. PHILLIPS AND ROBERT W. KEOWN

The action of diazomethane on 8-quinolinol gives, in addition to 8-methoxyquinoline, a red, ether-insoluble solid originally thought to have structure I.[1] Apparently without further experimentation Schenkel-Rudin[2] pointed out that structure II, corresponding to nitrogen methylation of 8-quinolinol, was more in accord with the polar properties of the compound. (For convenience this compound will be called diazoxine hereafter.) The following new experimental facts support the Schenkel-Rudin structure.



I



II

5,7-Dibromo-8-quinolinol reacts with diazomethane to give a product showing a similar absorption spectrum (with a bathochromic shift due to the weighting effect of bromine) to that of diazoxine (Fig. 1). Diazoxine is quantitatively dibrominated to give a substance spectrophotometrically identical to the diazomethane-5,7-dibromo-8-quinolinol product.

Determination of the absorption spectrum of diazoxine in solutions of *p*H 1–13 shows only two different structures, a nearly colorless hydrochloride (which was isolated and analyzed) having absorption maxima at 313, 323 and 365 mμ, and an orange

(1) Caronna and Sansone, *Gazz. chim. ital.*, **69**, 24 (1939).
(2) Schenkel-Rudin, *Helv. Chim. Acta*, **27**, 1456 (1944).

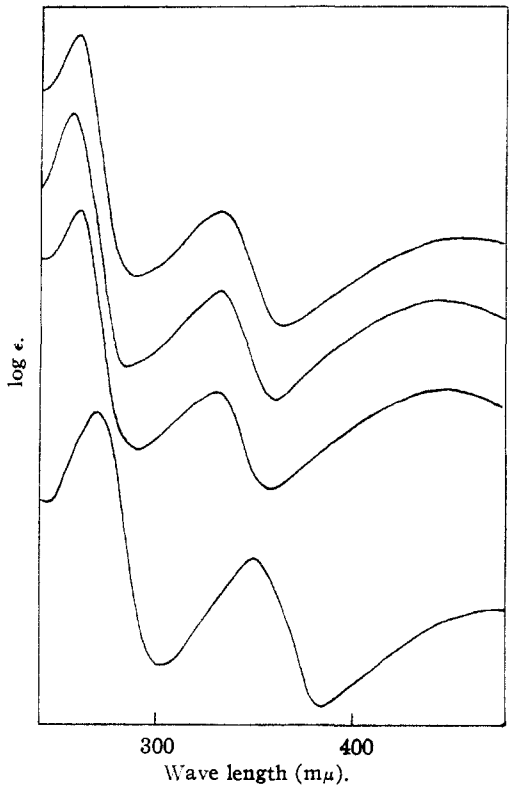
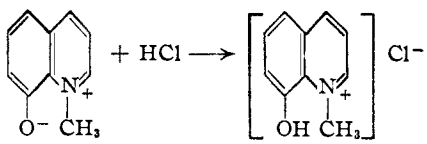


Fig. 1.—Absorption spectra of diazomethane-8-quinolinols addition products: from top to bottom, 8-quinolinol, 4-methyl-8-quinolinol, 3,4-dimethyl-8-quinolinol and 5,7-dibromo-8-quinolinol in 95% alcohol. The ordinates are shifted for each curve to prevent overlapping.

structure in basic solution having maxima at 345 and 445 mμ. The equilibrium for the presumed



structure change can be calculated from the measured extinctions (Table I); the substance is 50% in each form at a *p*H of 6.8.

TABLE I

CHANGE IN EXTINCTION OF DIAZOXINE WITH *p*H AT 460 mμ

*p*H	Extinction	*p*H	Extinction
1–2.5	0.005	7.0	0.660
4.8	.012	7.5	.860
5.6	.065	7.9	.940
6.5	.354	9.6–13	1.050
6.7	.472		

In solvents other than water diazoxine shows a variety of colors ranging from yellow to blue (Table II). These color changes might be expected as the result of solvate formation. The solubility of diazoxine in water and alcohols is quite large; the compound is slightly soluble (less than 12 mg./100 ml.) in ether and benzene.

TABLE II

ABSORPTION MAXIMA OF DIAZOXINE IN VARIOUS SOLVENTS

Solvent	Maximum, mμ	Solvent	Maximum, mμ
Hydrochloric acid	<400	Pyridine	550
Absolute alcohol	485	Dry benzene	590–600
Acetone	535–540	Dry ether	605–615

The change in color of solutions of diazoxine in alcohol upon addition of water suggests the use of this phenomenon for the analysis of alcohol–water mixtures (Table III). Pronounced changes in color are also observed in acetone solution on the addition of small amounts of water or benzene when a few tenths of a per cent. of absolute alcohol are added.

TABLE III

EXTINCTION AT 500 mμ OF DIAZOXINE (0.120 G./L.) IN ALCOHOL–WATER MIXTURES

Water, %	Extinction	Water, %	Extinction
1.25	0.800	68.8	0.456
3.12	.806	80.6	.394
9.40	.769	88.8	.363
20.0	.719	96.3	.331
30.0	.669	98.1	.325
41.3	.613	100	.300
49.4	.569		

The action of diazomethane on 3,4-dimethyl-8-quinolinol and 4-methyl-8-quinolinol gives compounds spectrophotometrically very similar to diazoxine (Fig. 1). A similar product was not obtained under the same conditions from 8-hydroxyquinaldine and a few other 2-substituted 8-quinolinols.

Diazoxine does not give a color with ferric ion in acid solutions.

Experimental

Preparation of Diazoxine.—A 100% or larger excess of an ether solution of diazomethane is added to an ether solution of 8-quinolinol and allowed to stand 24 hours. The precipitated product is removed by filtration and washed with ether. Traces of water in the ether gave a hydrated product; this probably explains the erratic yields obtained on successive trials; maximum yield 30%. Evaporation of the filtrate left a dark colored oil, presumably 8-methoxyquinoline.[1] The hydrate was analyzed for nitrogen (calcd. for $C_{10}H_9NO \cdot H_2O$: N, 7.9; found: N, 7.2), and brominated with standard bromate–bromide in acid solution (indicating a purity of 100.2 ± 1.3%). Attempted analysis for water by drying at 110° gave a result high by about 2% due to slight decomposition. Upon heating the compound darkens in color, melting with decomposition at 119°; further heating to 140° causes it to swell to about twice its volume.

The hydrochloride was prepared by adding an excess of hydrochloric acid to diazoxine and evaporating to dryness under reduced pressure. The hydrochloride melts with decomposition at 197°. *Anal.* Calcd. for $C_{10}H_9NO \cdot HCl$: Cl, 18.1. Found: Cl, 18.7.

The 5,7-dibromo derivative of diazoxine was obtained by adding the calculated amount of standard bromate–bromide solution to an acid solution of diazoxine and making alkaline with sodium hydroxide. The red-brown 5,7-dibromo derivative precipitates. The same compound is also obtained by treating 5,7-dibromo-8-quinolinol in ether with diazomethane; m.p. > 200°. The spectra in 0.1 *N* hydrochloric acid of samples prepared by both methods showed absorption maxima at 267 and 375 mμ.

Absorption Spectra.—All measurements were made with a Beckman model DU spectrophotometer using 1.00-cm. cells. Determinations of variations in absorption of diazoxine with *p*H were performed on solutions containing 0.128 g./l. Measurements were made from 320–700 mμ except where otherwise indicated. Diazoxine obeys Beer's law in 0.1 *N* acid or base over the concentration range employed here.

Acknowledgment.—The authors were supported in this work by a grant from the Research Corporation.

DEPARTMENT OF CHEMISTRY
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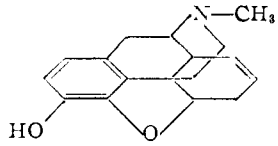
LOUISVILLE, KENTUCKY

RECEIVED MAY 21, 1951

Δ^7-Desoxymorphine

BY HENRY RAPOPORT AND ROBERT M. BONNER

The ready availability of Δ^7-desoxycodeine[1] led us to examine the possibility of preparing the morphine analog, Δ^7-desoxymorphine (I), by ether-cleavage.



Although the cleaving agents commonly employed in the morphine series, such as hydrogen bromide in glacial acetic acid, proved too drastic, heating with pyridine hydrochloride[2] gave good yields of the morphine compound. That no other change had taken place in the molecule was shown by re-etherification to Δ^7-desoxycodeine with diazomethane.

Preliminary testing of Δ^7-desoxymorphine was kindly carried out by Dr. Nathan B. Eddy[3] who reported "the LD_{50} is 90, the analgesic dose is 0.2, the onset of effect is very rapid (about five minutes), and the duration of effect is short (about 53 minutes). The comparable values for morphine are LD_{50} 539; analgesic dose, 1.70; onset of effect, 15 minutes; and duration of effect, 144 minutes."

Experimental

Δ^7-Desoxymorphine.—A mixture of 2.0 g. of Δ^7-desoxycodeine[1] and 6 g. of pyridine hydrochloride was placed in a bath at 220° and heated for six minutes in a nitrogen atmosphere, after which the reaction mixture was immediately cooled and treated with 25 ml. of water. Non-phenolic material was removed by ether extraction after the solution had been made alkaline with sodium hydroxide, and the ether extract was washed with water, dried over magnesium sulfate, and evaporated to give 1.2 g. (60%) of recovered Δ^7-desoxycodeine. The aqueous phase was adjusted to *p*H 8 by addition of hydrochloric acid, and the mixture was extracted with methylene chloride. Evaporation of the methylene chloride left 0.7 g. (37% yield based on original Δ^7-desoxycodeine or 92% yield based on unrecovered starting material) of phenolic material which was crystallized from benzene (0.1 g. in *ca.* 2 ml. of benzene). In order to free the compound from benzene which it retains tenaciously, it was slowly heated to 125° and sublimed at this temperature at 0.05 mm. Pure Δ^7-desoxymorphine (0.47 g., 62%) was thus obtained, m.p. 143–144°; $[\alpha]^{25}D$ −67.2° (*c* 1.31, ethanol).

Anal. Calcd. for $C_{17}H_{19}NO_2$: C, 75.8; H, 7.1. Found: C, 75.8; H, 7.0.

A sample dissolved in methanol was converted to Δ^7-desoxycodeine by treatment with ethereal diazomethane.

DEPARTMENT OF CHEMISTRY AND RADIATION LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

RECEIVED JUNE 25, 1951

(1) H. Rapoport and R. M. Bonner, THIS JOURNAL, 73, 2872 (1951).
(2) V. Prey, *Ber.*, 74, 1219 (1941).
(3) National Institutes of Health, Bethesda 14, Maryland. Doses are expressed in milligrams of base per kilogram of body weight for subcutaneous administration to mice.

A Solvent Extraction Procedure for Purifying Streptomycin

BY H. W. RHODEHAMEL, JR., W. B. FORTUNE AND S. L. MCCORMICK, JR.

The insolubility of streptomycin base and of mineral-acid salts of streptomycin in common organic solvents immiscible with water has precluded isolation or purification of streptomycin by simple solvent extraction procedures. Several solvent extraction systems have been reported[1,2] in which streptomycin has been solubilized in organic solvents by the formation of salts of streptomycin with non-polar organic acids. Other basic organic impurities are likewise solubilized, however, and, in consequence, little purification is achieved.

It has been found that water-immiscible, primary liquid alkyl or aralkyl amines have the ability to extract streptomycin from water solutions in satisfactory yields with a high degree of selectivity and with considerable purification. Reactions postulated for this selective extraction are the formation of an amine soluble combination of a Schiff base, or alcohol–ammoniate type linkage between the carbonyl group of the streptomycin molecule and the primary amine group. Such postulations gain support by the facts that dihydrostreptomycin is not extracted by this system, and that secondary and tertiary amines are ineffective in extracting streptomycin.

With suitable amines, streptomycin activity has been extracted efficiently from aqueous streptomycin solution of virtually any degree of purity, including filtered fermentation broths. The streptomycin solution must be on the basic side of neutrality for the extraction to take place. Except in cases of buffered solutions, the amine itself will raise the *p*H sufficiently. For efficient single-stage extraction, a high inorganic salt concentration in the streptomycin water phase is necessary. Since certain initial isolation steps for streptomycin tend to give concentrates of streptomycin high in salt content, for example, eluates of streptomycin activity from ion-exchange resins, this requirement for a high salt concentration in the aqueous phase is not necessarily undesirable.

The streptomycin may be recovered from the amine phase by extracting the latter with water and a water-immiscible solvent in which the amine used is soluble. For satisfactory recovery, it is necessary to have a streptomycin concentration in the amine phase equivalent to 150–300 mg. of streptomycin base per ml. This may be accomplished either in the original extraction by using suitable volumes of the amine phase or by concentration of the amine phase after extraction of and separation from the aqueous phase. Chloroform and amyl acetate have been found effective as the water-immiscible solvent to be used in conjunction with water to recover the streptomycin from the amine phase. The aqueous phase resulting from the mixture of chloroform (or amyl acetate), amine and water will contain substantially all the streptomycin originally present in the amine phase.

(1) E. Titus and J. Fried, *J. Biol. Chem.*, 168, 393 (1947).
(2) U. S. Patents 2,537,933 (Jan. 9, 1951) and 2,537,934 (Jan. 9, (1951).