of di-DNP-L-lysine and 5 moles of N^{ϵ}-DNP-L-lysine per mole of lysozyme confirms the previous analyses of Green and Schroeder[5] and of Lewis, *et al.*[3]

TABLE I

RECOVERY OF N^{ϵ}-DNP-L-LYSINE AND DI-DNP-L-LYSINE FROM DNP-LYSOZYME AND XANTHYL-DNP-LYSOZYME

Sample	Quantity analyzed,[a] μM	N^{ϵ}-DNP-L-lysine recov.[b] μM	%	Di-DNP-L-lysine recov.[b] μM	%
DNP-Lysozyme	1.20	3.37	56.0	0.94	78.0
Xanthyl-DNP-lysozyme	1.34	6.10	91.0	1.31	97.5

[a] Calculated from determination of primary amide groups.[6,7] [b] Calculated from absorbancy at 3460 Å. in glacial acetic acid.

The increased recovery of the DNP-lysines after xanthylation is probably due to the formation of dixanthyltryptophan in the intact protein. This is also indicated by the formation of the characteristic purple color of dixanthyltryptophan in these solutions. Data which indicate that dixanthyltryptophan itself is non-destructive of DNP-alanine under protein hydrolysis conditions is included in Table II. A 56% recovery of DNP-DL-alanine was obtained in the presence of tryptophan. This was increased to a recovery of 82% in the presence of dixanthyltryptophan and 85% in the presence of xanthyllysozyme and in the control. These results suggest that xanthylation of other proteins after reaction with DNFB will result in higher recoveries of DNP-amino acids in acid hydrolysates of proteins.

TABLE II

EFFECT OF ADDED COMPOUNDS ON THE RECOVERY OF DNP-DL-ALANINE FROM ACID SOLUTIONS

Compound[a]	Mg.	Recovery, %
Control	..	85
Tryptophan	10	56
Dixanthyltryptophan	30	82
Xanthyllysozyme	100	85

[a] These quantities provide approximately 10 moles of tryptophan per mole of DNP-DL-alanine.

Experimental

DNP-Lysozyme was prepared by the method described by Sanger[7] for DNP-insulin. Lysozyme (100 mg.) was treated with DNFB (400 mg.) in bicarbonate buffer and 85 mg. of DNP-lysozyme was isolated.

Xanthyl-DNP-lysozyme was prepared by dissolving DNP-lysozyme (100 mg.) and xanthydrol (112 mg.) in 10 ml. of 90% acetic acid. At the end of one hour at room temperature the product was precipitated with ether and washed three times with ether at the centrifuge. The xanthyl-DNP-lysozyme (65 mg.) was dried over calcium chloride and paraffin.

Isolation and determination of N^{ϵ}-DNP-L-lysine and di-DNP-L-lysine from DNP-lysozyme and xanthyl-DNP-lysozyme were carried out according to Porter.[8] Synthesis of the two DNP-lysines which were used as standards was also accomplished by the method of Porter. Absorbancy measurements of the DNP-lysines were made with a Beckman DU spectrophotometer at 3460Å.

Protein concentrations were determined by primary amide group analyses. The protein solutions were autoclaved for one hour in 2 *N* H_2SO_4 at 20 lb. pressure, after which the ammonia was determined as described by Porter.[8]

DNP-DL-alanine was synthesized by the method of Porter.[8] For the stability study DNP-DL-alanine (1.0 mg.) was refluxed twenty-four hours in 12 *N* HCl in the presence of tryptophan (10 mg.), dixanthyltryptophan (30 mg.) and xanthyllysozyme (100 mg.). The DNP-DL-alanine in the solution was chromatographed over silica gel according to Porter[8] and determined photometrically at 4000 Å.

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Amebacidal Agents. II. 5-Acyl- and 5-Alkyl-7-dialkylaminomethyl-8-quinolinols

BY WILLIAM H. EDGERTON AND J. H. BURCKHALTER

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Interesting amebacidal activity has been reported in a group of Mannich base derivatives of halogenated quinolinols.[1] A similar series of alkylated quinolinols was synthesized following the initial observation of activity. The intermediate 5-acyl-8-quinolinols were prepared by means of the Friedel–Crafts reaction using 8-quinolinol.[2] Reduction of these ketones to the 5-alkyl-8-quinolinols was accomplished by catalytic hydrogenation with 5% palladium-on-charcoal.[2] This reaction was extended with great difficulty to the preparation of several long-chain acylated quinolinols. Reduction of the long-chain acylated quinolines was confirmed by the disappearance of the characteristic infrared absorption band at 5.96 μ.[3]

When the Mannich reaction with 8-hydroxy-5-quinolyl methyl ketone,[2] was attempted, using two molar equivalents of amine and paraformaldehyde only one basic side chain was introduced. Infrared data strongly suggest that the piperidyl methyl group entered the aromatic nucleus at position 7 rather than the α-position of the acetyl substituent.[4] Compound VI, Table I, and 5-chloro-7-(1′-piperidylmethyl)-8-quinolinol dihydrochloride[5] possess similar infrared absorption patterns with bands at 11.6, 12.10, 12.42, 12.75 μ, and 11.66, 12.24, 12.54, 12.75 μ, respectively. Both spectra differ greatly in this region from the spectra of several 5-substituted 8-quinolinols.

The amebacidal activity[6] of these compounds was observed to decrease with increasing molecular weight.

Experimental

8-Hydroxy-5-quinolyl Octyl Ketone (X).—Anhydrous aluminum chloride (120 g.) was added in small portions with cooling to a mixture of 70.5 g. (0.40 mole) of pelargonyl chloride and 45.0 g. (0.31 mole) of 8-quinolinol in 200 g. of nitrobenzene. The mixture was heated at 75° for 16 hours.

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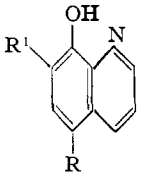
(5) Unpublished work.

(6) Amebacidal activity data were determined by Dr. Paul Thompson and staff of these laboratories.

TABLE I

5-ACYL- AND 5-ALKYL-8-QUINOLINOLS



Compound No.	R	R¹	M.p.,[a] °C.	Yield, %	Formula	Analyses,[b] % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found
I	Methyl[c]	Diethylaminomethyl	174	70	$C_{15}H_{20}N_2O\cdot HBr$	55.38	55.67	6.31	6.31
II	Methyl	4-Phenyl-1-piperazylmethyl	156	91	$C_{21}H_{23}N_3O$	75.65	75.96	6.95	7.30
III	Methyl	1-Piperidylmethyl[d]	268	97	$C_{16}H_{20}N_2O\cdot 2HBr$	45.95	46.24	5.34	5.35
IV	Methyl	4-Morpholinylmethyl	99	73	$C_{15}H_{18}N_2O_2$	69.74	69.76	7.02	7.30
V	Methyl	Ethyl-β-hydroxyethylaminomethyl	200	56	$C_{15}H_{20}N_2O_2\cdot 2HCl$	54.06	54.34	6.66	6.93
VI	Acetyl	1-Piperidylmethyl	208	67	$C_{17}H_{20}N_2O_2$	71.80[e]	71.72	7.09	7.36
VII	Ethyl	1-Piperidylmethyl	242	95	$C_{17}H_{22}N_2O\cdot HBr$	58.12	58.17	6.60	6.65
VIII	Benzoyl	1-Piperidylmethyl	251	81	$C_{22}H_{22}N_2O_2\cdot 2HBr$	51.98	52.24	4.76	5.11
IX	Benzyl	1-Piperidylmethyl	275	56	$C_{22}H_{24}N_2O\cdot 2HBr$	53.45	53.09	5.30	5.50
X	Pelargonyl		67	17[f]	$C_{18}H_{28}NO_2$	75.76	75.92	8.12	8.09
XI	Nonyl		217	16	$C_{18}H_{25}NO\cdot HBr$	61.31	61.27	7.44	7.57
XII	Palmitoyl		223	11	$C_{25}H_{37}NO_2\cdot HBr$	64.64	65.07	8.25	8.33
XIII	Palmitoyl	1-Piperidylmethyl	167	54	$C_{31}H_{48}N_2O_2\cdot HBr$	66.30	67.09	8.79	9.06
XIV	Hexadecyl[g]		63	63					

[a] Melting points are not corrected. Salts melt with decomposition. [b] Analyses are by Mr. Charles Childs and staff of these laboratories. [c] 5-Methyl-8-quinolinol was prepared by the method of E. Noelting and E. Trautmann, *Ber.*, **23**, 3666 (1890). [d] 1-Phenylpiperazine was supplied by Dr. Robert F. Parcell of these laboratories. [e] Calcd.: N, 9.85. Found: N, 10.04. [f] Yield based on hydrochloride. [g] Although an analytical sample could not be obtained, the characteristic infrared absorption band of the ketone moiety is missing.

After 20 hours at room temperature, the dark mixture was decomposed carefully with a slurry of dilute hydrochloric acid and ice. The nitrobenzene was steam distilled. The thick residue was neutralized with strong alkali until faintly acid to *p*H paper. The filtered reaction mass was extracted with several portions of acetone. The acetone solution was saturated with hydrogen bromide to yield 19 g. (17%) of yellow solid, m.p. 229–231° (dec.).

For analysis, a sample was converted to the free base by neutralizing an aqueous suspension with alkali extracting the dried solid with ether and recrystallizing the light brown crystals several times from ethanol; m.p. 66–67°.

The similar pentadecyl ketone (XII) was prepared in like manner. The ketones were reduced catalytically by the method of K. Rosenmund and G. Karst.[3]

5-Acyl- and 5-Alkyl-7-dialkylaminomethyl-8-quinolinols.— A previously heated solution of molar equivalents of amine and paraformaldehyde in ethanol was added to a solution of the quinolinol in ethanol. After a brief reflux period (1–2 hours), the solution was either concentrated and cooled to separate the product or diluted with dry ether and acidified with dry hydrogen bromide gas to separate the salt. The solid was then purified by several recrystallizations from ethanol or isopropyl alcohol.

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Additional Evidence on the Enzymatic Transformation of Histidine into Glutamic Acid

BY J. P. FOURNIER AND L. P. BOUTHILLIER

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By the use of heavy nitrogen as a tracer element, Tabor and his collaborators[1] demonstrated conclusively that the γ-nitrogen of histidine appears in the amino group of the glutamic acid resulting from the enzymatic degradation of the former. They have also presented direct evidence that the initial step in the breakdown of histidine is its deamination to the formation of urocanic acid. Their findings are thus in agreement with the theory formulated by Sera and his co-workers,[2] Takeuchi[3] and Oyamada,[4] whereby histidine would be biologically degraded to urocanic acid, formylisoglutamine, isoglutamine and glutamic acid.

We wish to report here some experimental data which provide additional support to the above theory. Freshly prepared liver homogenates were incubated in presence of carboxyl-C^{14}-labeled DL-histidine. Two-dimensional paper partition chromatograms (solvent mixtures: water-saturated-phenol + HCN + 0.3% NH_3 and water-saturated *n*-BuOH + glacial AcOH) of the original incubation mixtures, as compared to incubation blanks without histidine, revealed upon treatment with ninhydrin the marked intensification of only one spot, that corresponding to glutamic acid. The latter was further identified by the mixed spot technique. The biologically formed glutamic acid was then isolated with carrier, and recrystallized several times to constant radioactivity in the form of its calcium salt. Samples of the regenerated glutamic acid were decarboxylated (α-COOH) by treatment with ninhydrin and the activity of the evolved carbon dioxide was measured. The results appear in Table I. Since our data indicate that only a small fraction of the total activity contained in the glutamic acid was present in the α-carboxyl group of the molecule, it seems reasonable to assume that the radioactivity was concentrated almost entirely in the γ-carboxyl carbon. The conclusion is reached that the α- and γ-carboxyl carbons of the

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