After boiling off the acetone, 500 ml of ice water was added and the solution was saturated with NaCl. The reaction mixture was extracted (Et_2O) and the extract was dried (Na_2CO_3). Stripping off the ether left a brown viscous residue. Trituration of the residue with MeOH yielded 4.2 g (31%) of IV, mp 121.5–122.5° (MeOH).

The N-phenylpiperazines listed in Table I were synthesized by analogous procedures.

1-(2-Bromo-4,5-dimethoxyphenyl)-4-(2-methyl-4,5-dimethoxyphenyl)piperazine.—A solution of 31 g (0.05 mole) of II ditosylate and 25 g (0.15 mole) 4-methyl-5-aminoveratrole in 1 l. of 50% Me_2CO–H_2O was refluxed for 2 days. A solid which precipitated during the course of the reaction was filtered. After washing (Et_2O) and recrystallization (*i*-PrOH), it gave 4.2 g of white glistening flakes.

1-(2-Bromo-4,5-dimethoxyphenyl)-4-tetramethylenepiperazinium Chloride.—A solution of III (14 g) and 20 ml of pyrrolidine in 150 ml of THF was refluxed for 2 days. Filtration of the cooled reaction mixture gave 16 g of a tan solid. Extraction with hot *i*-PrOH left the piperazinium salt as a white solid (8.9 g).

The piperazinium salts listed in Table II were prepared in a like manner.

Preparation of Some Sulfonamide and Diaminodiphenyl Sulfone Analogs of 1,4-Naphthoquinone[1]

F. I. CARROLL, KENNETH H. DUDLEY, AND H. WAYNE MILLER

Chemistry and Life Sciences Laboratory, Research Triangle Institute, Research Triangle Park, North Carolina 27709

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Although the activity of sulfonamide drugs against human malaria has been disappointing, the capacity of these drugs to potentiate the activity of other antimalarial agents has renewed an interest in this class of compounds.[2,3] Zbinden has pointed out the striking differences in the lipid solubility and pK_a of various sulfonamides and the consequent influences of these differences on the kinetics of drug absorption and excretion.[4] We have prepared a series of sulfonamides which incorporate a naphthoquinone residue at the N^4 nitrogen[5] for evaluation of antimalarial activity.[6] The naphthoquinone analogs reported herein were prepared from a series of sulfonamides which show a spectrum of variances in their physical dispositions.[4]

Table I summarizes the synthesis of the sulfonamide and diaminodiphenyl sulfone analogs of 1,4-naphthoquinone. N^4-(1,4-Naphthoquinonyl)sulfanilamide (Ia) and N-(1,4-naphthoquinonyl)-α-amino-*p*-toluenesulfonamide (Ib) were prepared by the condensation of the appropriate sulfonamide with 2-hydroxy-1,4-naphthoquinone in 80% AcOH (procedure A, Table I). Calandra and Adams reported that the treatment of 2,3-dichloro-1,4-naphthoquinone with sulfadiazine in refluxing ethanol catalyzed by N,N-diethylaniline gave N^4-(3-chloro-1,4-naphthoquinonyl)sulfadiazine (Ic).[7] Attempts to repeat this preparation were unsuccessful. Tlc[8] showed very faint orange spots that could be attributed to the expected product, but the yield was so small that isolation was impossible. The reaction of 2,3-dichloro-1,4-naphthoquinone with sulfadiazine in DMF catalyzed by K_2CO_3 (procedure C, Table I) also gave only trace amounts of the desired product. The N^4-(3-chloro-1,4-naphthoquinonyl)sulfadiazine (Id) could be obtained, however, by treating 2,3-dichloro-1,4-naphthoquinone with sulfadiazine in DMSO catalyzed by K_2CO_3 (procedure B, Table I). The product obtained had mp 298–300° compared to mp 256° reported by Calandra and Adams. However, the analysis and ir spectra are in agreement with the assigned structure. The naphthoquinone sulfonamides Id and Ie could be obtained by procedures D, C, or B, but again procedure B was the method of choice. The analogs If–j were prepared in satisfactory yield by the procedure of Calandra and Adams (procedure D, Table I). The 3-chloronaphthoquinonyl derivative Ii of diaminodiphenyl sulfone (DDS) is the product of the reaction using 1:3 and 3:1 ratios, respectively, of 2,3-dichloro-1,4-naphthoquinone and DDS. The identity of the product from these reactions was established by mixture melting point, tlc, and comparison of ir spectra. The analytical data were in agreement with the formula $C_{22}H_{15}ClN_2O_4S$, and the compound gave a mono-N-formyl (Ik) and mono-N-acetyl (Il) derivative. The DDS derivatives were of interest since many chloroquine-resistant strains of malaria parasites did not show cross resistance to DDS.[9]

The sulfonamide and DDS analogs of 1,4-naphthoquinone, have been tested against *Plasmodium berghei* in mice by Dr. Leo Rane.[10,11] These compounds exhibited negligible antimalarial activity. N^4-(3-Chloro-1,4-naphthoquinonyl)sulfadiazine (Ic) which showed a 2.3-day extension of survival time at a dose of 80 mg/kg and a 2.9-day extension at 320 mg/kg was the most active compound.[12] Deaths occurring on days 2–5 after infection are attributed to drug action and counted as "toxic" deaths. Control animals do not die before day 6. According to this criterion, these compounds were not toxic at a dose of 640 mg/kg.

(1) This investigation was carried out under Contract No. DA-49-193-MD-2862 with the Department of the Army and the U. S. Army Research and Development Command. This paper is Contribution No. 390 from the Army Research Program on Malaria.

(2) (a) D. C. Martin and J. D. Arnold, *J. Am. Med. Assoc.*, **203**, 476 (1968); (b) P. J. Bartelloni, T. W. Sheehy, and W. D. Tigertt, *ibid.*, **199**, 173 (1967).

(3) Using a combination of 2-sulfanilamido-3-methoxypyrazine (Sulfalene) and 2,4-diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine (trimethoprim), Martin and Arnold[2] effected radical cures against a normal, as well as a chloroquine–quinine–pyrimethamine-resistant *Plasmodium falciparum*.

(4) G. Zbinden, "Molecular Modifications in Drug Design," Advances in Chemistry Series, No. 45, American Chemical Society, Washington, D. C., 1964, p 25.

(5) In a uniform nomenclature of sulfonamide drugs, the sulfonamide nitrogen is designated N^1 and the aromatic amino nitrogen N^4.

(6) Molecular modification by N^4 acetylation, alkylation, arylation, or arylsulfonylation renders the sulfonamide drugs, as a class, inactive. Two exceptions to this N^4 substitution generalization are N^4-phthalylsulfathiozole and N^4-succinylsulfathiozole.[4] In addition Calandra and Adams[7] found that the incorporation of the 2-(3-chloro-1,4-naphthoquinonyl) group at the N^4 position of certain sulfanilamides gave analogs which were active inhibitors of acid production by bacteria in the oral cavity. Fieser and colleagues have shown that certain 1,4-naphthoquinones have considerable antimalarial activity; see L. F. Fieser, J. P. Schirmer, S. Archer, R. R. Larenz, and P. I. Pfaffenbach, *J. Med. Chem.*, **10**, 513 (1967), for a review of this work.

(7) J. C. Calandra and E. C. Adams, Jr., *J. Am. Chem. Soc.*, **72**, 4804 (1950).

(8) Tlc on 25 × 75 mm microscope slides covered with Brinkmann silica gel HF, eluent C_6H_6–EtOH–AcOH (9:1:1).

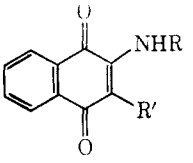
(9) A. B. G. Laing, *J. Trop. Med. Hyg.*, **63**, 25 (1960).

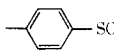
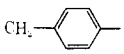
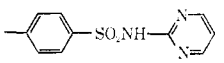
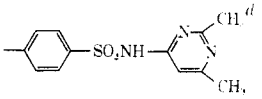
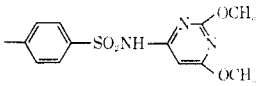
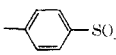
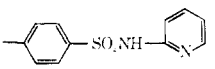
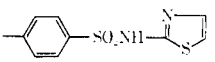
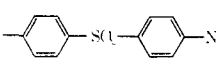
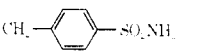
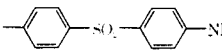
(10) T. S. Osdene, P. B. Russell, and L. Rane, *J. Med. Chem.*, **10**, 431 (1967).

(11) Printout interpretation for rodent antimalarial test results, Walter Reed Army Institute of Research.

(12) Antimalaria test results were supplied through the courtesy of Dr. David P. Jacobus of the Walter Reed Army Institute of Research.

TABLE I
SULFONAMIDE AND DDS ANALOGS OF 1,4-NAPHTHOQUINONE



No.	R	R′	Method[a]	Recrystn solvent	Yield,[b] %	Mp, °C	Formula	Analyses[14]
a		H	A	THF–H_2O	41	293–298 dec	$C_{16}H_{12}N_2O_4S$	C, H, N, S
b		H	A	THF–H_2O	35	240–246 dec	$C_{17}H_{14}N_2O_4S$	C, H, N, S
c		Cl	B	THF	8	298–300 dec[c]	$C_{20}H_{13}ClN_4O_4S$	C, H, Cl, N, S
d		Cl	B, C D	THF–H_2O	28, 12 6	155[e]	$C_{22}H_{17}N_4ClO_4S$	C, H, Cl, N, S
e		Cl	B, C D	Me_2CO–H_2O	39, 20 6	222–225	$C_{22}H_{17}ClN_4O_6S$	C, H, Cl, N, S
f		Cl	D	DMF–H_2O	25	313–315 dec	$C_{16}H_{11}ClN_2O_4S$	C, H, Cl, N, S
g		Cl	D	THF–H_2O	22	266–271 dec[f]	$C_{21}H_{14}ClN_3O_4S$	C, H, Cl, N, S
h		Cl	D	THF–H_2O	28	278–281 dec[g]	$C_{19}H_{12}ClN_3O_4S_2$	C, H, Cl, N, S
i		Cl	D	EtOH	72	239.5–240.5 dec	$C_{22}H_{15}ClN_2O_4S$	C, H, Cl, N, S
j		Cl	D	THF–H_2O	35	242–244.5 dec	$C_{17}H_{13}ClN_2O_4S$	C, H, Cl, N, S
k		Cl	E	DMF–H_2O	90	244–248 dec	$C_{23}H_{15}ClN_2O_5S$	C, H, Cl, N, S
l		Cl	E	THF–H_2O	73	306–312 dec	$C_{24}H_{17}ClN_2O_5S$	C, H, Cl, N, S

[a] An example of each procedure is given in the Experimental Section: A, 2-hydroxy-1,4-naphthoquinone + H_2NR in 80% AcOH; B, 2,3-dichloro-1,4-naphthoquinone + H_2NR in DMSO containing K_2CO_3; C, 2,3-dichloro-1,4-naphthoquinone + H_2NR in DMF containing K_2CO_3; D, 2,3-dichloro-1,4-naphthoquinone + H_2NR in EtOH containing $PhNEt_2$; E, formylation with formic acid or acetylation with Ac_2O. [b] Based on pure compound isolated. [c] Lit.[7] mp 256°. [d] The acetate salt prepared by recrystallization of the free base from HOAc had mp 232–236°. *Anal.* ($C_{24}H_{21}ClN_4O_6S$) C, H, N, S, Cl. [e] Resolidified and melted at 232–235°. [f] Lit.[7] mp 262° dec. [g] Lit.[7] mp 279–281° dec.

Experimental Section[13,14]

N-(1,4-Naphthoquinonyl)-α-amino-*p*-toluenesulfonamide (Method A, Table I).—This procedure used an equivalent amount of Et_3N for neutralization of the HCl salt of α-amino-*p*-toluenesulfonamide. The Et_3N was omitted in cases where the free base of the sulfonamide was employed. A mixture of 5.0 g (28.7 mmoles) of 2-hydroxy-1,4-naphthoquinone, 6.4 g (28.7 mmoles) of α-amino-*p*-toluenesulfonamide hydrochloride, and 2.9 g (28.7 mmoles) of NEt_3 in 200 ml of 80% AcOH was heated on the steam bath for 18 hr, after which a dark solution had formed. The hot solution was filtered and a 100-ml portion of hot H_2O was added to the filtrate, whereupon a copious crop of yellow-gold plates separated. The product was collected and washed (80% AcOH, EtOH, Et_2O) and then vacuum dried; yield 3.38 g. The compound was recrystallized by adding an equal volume of hot H_2O to its hot solution in 250 ml of 80% THF; yield 2.88 g, mp 242–246° dec. The compound moved as one zone when a tlc was eluted in C_6H_6–EtOH–HOAc (9:1:1). Other examples are given in Table I.

Sulfonamide and DDS Analogs of N⁴-(3-Chloro-1,4-naphthoquinone) (Method B, Table I).—A mixture of 0.02 mole of 2,3-dichloro-1,4-naphthoquinone, 0.02 mole of sulfonamide or DDS, and 0.02 mole of anhydrous K_2CO_3 in 40 ml of dry DMSO (distilled from CaH_2) was stirred at room temperature until a tlc indicated that reaction was completed. The reaction mixture was diluted with H_2O. The resulting precipitate was separated by filtration, vacuum dried, and recrystallized from the appropriate solvent. See Table I.

N-(3-Chloro-1,4-naphthoquinonyl)-α-amino-*p*-toluenesulfonamide (Method D, Table I).—In preparations where the free base of the sulfonamide was employed, an equivalent amount of $PhNEt_2$ was added to take up the liberated HCl. In this particular experiment, where the hydrochloride salt of the sulfonamide was employed, 2 equiv of $PhNEt_2$ were added to the solution.

A mixture of 5.0 g (22.0 mmoles) of 2,3-dichloro-1,4-naphthoquinone, 5.0 g (22.5 mmoles) of α-amino-*p*-toluenesulfonamide hydrochloride, and 6.7 g (45.0 mmoles) of $PhNEt_2$ in 500 ml of absolute EtOH was stirred and heated under reflux for 18 hr. At the end of the reaction period, a copious crop of orange crystalline product had separated from the hot solution. The solution was filtered while hot, and the collected product (3.65 g) was washed thoroughly with EtOH and Et_2O. The product was recrystallized by solution in 900 ml of boiling 80% THF, filtration, and adding 450 ml of hot H_2O; yield 2.85 g, mp 242–244° dec. The compound moved as one zone on a tlc.[8] The analysis and other examples are given in Table I.

N-Formyl-N′-(3-chloro-1,4-naphthoquinonyl) Diaminodi-

(13) Melting points were determined on a Kofler hot stage microscope using a calibrated thermometer. Ir spectra (which were as expected) were measured with a Perkin-Elmer 221 spectrophotometer using KBr disks. Microanalyses were performed by MicroTech Laboratories, Skokie, Ill.

(14) Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

phenyl Sulfone.—A solution of 2.5 g (5.7 mmoles) of N-(3-chloronaphthoquinonyl) diaminodiphenyl sulfone in 40 ml of 97% HCO_2H was heated under reflux for 2.0 hr, an equal volume of H_2O was added to the hot solution, and the bright orange crystalline precipitate was collected and washed (H_2O, MeOH, Et_2O); yield 2.6 g, mp 244–248° dec. The compound was recrystallized by solution in 100 ml of DMF (dissolved at room temperature) and adding an equal volume of H_2O. The product (2.4 g) separated as glistening orange platelets, mp 244–248°. See Table I.

N-Acetyl-N'-(3-chloro-1,4-naphthoquinonyl) Diaminodiphenyl Sulfone.—N-(3-Chloronaphthoquinonyl) diaminodiphenyl sulfone (0.3 g, 1.25 mmoles) was suspended in 20 ml of Ac_2O and then warmed on the steam bath. The red crystalline suspension dissolved and an orange crystalline product precipitated. After cooling to room temperature, the crystals were collected by filtration and washed (MeOH, Et_2O); yield 0.29 g. The crystals were recrystallized from a THF and H_2O mixture to give 0.27 g of product. See Table I.

Acknowledgment.—The authors are grateful to Dr. M. E. Wall, Director of this laboratory, for his kind encouragement and support of this work.

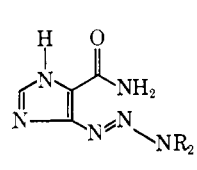
Single Crystal Studies of Chemotherapeutic Agents. I. The Structure of 1-(2-Chloroethyl)-3-(5-carbamoylimidazol-4-yl)-Δ^2-1,2,3-triazolinium Chloride[1]

D. J. ABRAHAM,[2] J. S. RUTHERFORD, AND R. D. ROSENSTEIN

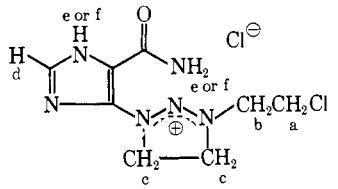
Department of Medicinal Chemistry, School of Pharmacy and the Crystallography Laboratory, University of Pittsburgh, Pittsburgh, Pennsylvania 15213

Received September 13, 1968

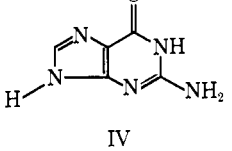
The nitrogen mustard 5(4)-[3,3-bis(2-chloroethyl)-1-triazeno]imidazole-4(5)-carboxamide (I) is a promising anticancer agent.[3] Other related triazenes, particularly the dimethyl compound II, also show anticancer activity to a lesser extent.[4] Unfortunately, the nitrogen mustard I undergoes a spontaneous



I, R = CH_2CH_2Cl
II, R = CH_3



III



IV

transformation at room temperature in the solid state to form an inactive isomer, which was recognized as a quaternary ammonium chloride by Shealy, *et al.*[5]

They further suggested that it might be an aziridinium, piperazinium, or *v*-triazolinium salt. We wish to report the identification of the transformation product, by X-ray crystal structure analysis as well as nmr and mass spectral studies, as 1-(2-chloroethyl)-3-(5-carbamoylimidazol-4-yl)-Δ^2-1,2,3-triazolinium chloride (III). This shows that the transformation reaction is a novel means of formation of the *v*-triazoline ring system.

Bond lengths and angles between nonhydrogen atoms at the present stage of refinement (R = 0.10) are shown in Figure 1. The two rings, amide group, and α carbon of the chloroethyl group are roughly coplanar, the maximum deviation of any atom from the plane being 0.22 Å. The bond lengths and angles in the triazolinium ion show that it has the symmetrical resonance-stabilized form. The chloroethyl group has a *gauche* conformation. The hydrogen atoms have been located in a three-dimensional difference synthesis, and their positions confirm the tautomeric form ascribed to the imidazole ring, and the existence of an intramolecular N–H···N hydrogen bond of length 2.98 Å between the amide N and the N^2 of the triazoline ring.

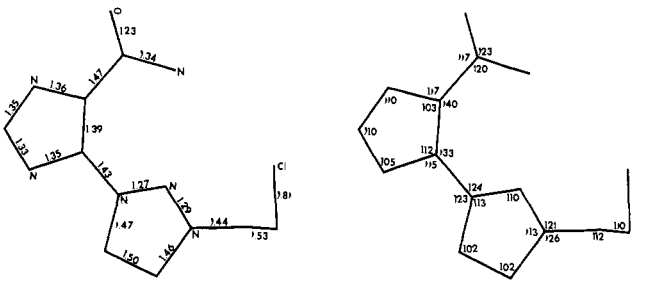


Figure 1.—Bond lengths and angles in the 1-(2-chloroethyl)-3-(5-carbamoylimidazol-4-yl)-Δ^2-1,2,3-triazolinium ion.

The nmr spectrum agrees well with this structure, both in the ratios of the various types of hydrogens and in the observed splittings. The fact that all CH_2 protons occur downfield from the usual CH_2 range is in accord with this structure, since b and c are next to a positive N, while protons of type a are adjacent to Cl.

This investigation further allows us to draw inferences concerning the mode of action of the drug, based on the stereochemistry of the inactive compound. The substituent groups on the imidazole ring are almost coplanar with it, and so arranged that the amide N and N^2 of the triazoline ring face each other at a distance of about 3 Å. An intramolecular H bond between these two atoms holds the molecule in this configuration. If we assume that this arrangement also occurs in the parent compound, the structural similarity to guanine (IV) becomes immediately apparent. The C(2)–N(1) bond of guanine has been replaced by this N–H···N hydrogen bond. The peripheral pattern of H-bond donors and acceptors, required by guanine's role in the nucleic acids and determining the specificity of enzymes, remains unchanged except for the modification of N^2 to a nitrogen mustard. The fact that the anticancer activity remains, although reduced, when these 2-chloroethyl groups are replaced by other substituents, supports the conclusion that the activity is related to the structure of the remainder of the molecule. The over-all implication is that I may bind,

(1) This research was supported by contract PH43-67-1186 from the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Bethesda, Md.

(2) Joint member of both the Department of Medicinal Chemistry and the Crystallography Laboratory.

(3) Y. F. Shealy and C. A. Krauth, *Nature*, **210**, 208 (1966).

(4) Y. F. Shealy, J. A. Montgomery, and W. R. Lafter, Jr., *Biochem. Pharmacol.*, **11**, 674 (1962).

(5) Y. F. Shealy, C. A. Krauth, L. B. Holum, and W. E. Fitzgibbon, *J. Pharm. Sci.*, **57**, 83 (1968).