hydrolysate (6 *N* HCl, 20 hr, 110°) gave the following molar ratios: Lys 1.0, NH_3 1.8, Asp 1.1, Glu 1.1, Pro 1.0, Cys 0.8, mixed disulfide[7] of Cys and β-mercaptopropionic acid 0.3, Tyr 1.0, and Phe 1.0. *Anal.* ($C_{46}H_{66}N_{12}O_{11}S_2 \cdot C_2H_4O_2 \cdot 2H_2O$) C, H, N.

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A Bis-*N,O*-diacetylhydroxylamine Analog of Diaminodiphenyl Sulfone Possessing Antimalarial Activity

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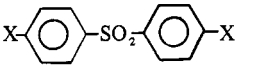
4,4′-Bis(*N,O*-diacetylhydroxylamino)diphenyl sulfone (TAHDS) was prepared and found to have good prophylactic activity against *Plasmodium berghei*. It is significantly more active than is 4,4′-diaminodiphenyl sulfone (DDS). 4,4′-Dihydroxylaminodiphenyl sulfone (DHDS), a possible metabolite of DDS, was prepared and found to be essentially inactive. The antimalarial activity of TAHDS does not appear to be the result of hydrolysis or metabolic conversion to DDS. TAHDS also possesses antileprotic activity.

4,4′-Diaminodiphenyl sulfone (DDS), used extensively for the treatment of leprosy[1] and of dermatitis herpetiformis,[2] has, since 1941, been known to possess antimalarial activity also.[3] No great interest in its antimalarial activity was aroused until 1960 when it was reported that leprotic patients undergoing treatment with DDS were protected from *falciparum* and to some extent *quartan* malaria.[4] Subsequent work by Elslager and coworkers[5] led to the development of repository combinations of DDS derivatives with cycloguanil or pyrimethamine.

The current regimen for prevention of chloroquine-resistant *falciparum* malaria is administration of DDS along with the standard antimalarial drugs chloroquine and primaquine. However, the use of DDS is undesirable due to the fact that it has a very short lifetime as a consequence of rapid metabolism, it causes methemoglobinemea,[6,7] and, particularly in combination with primaquine, DDS causes hemolysis.[8] The high doses of DDS required to compensate for rapid elimination from the body generates higher initial blood levels than are needed to suppress malaria and causes toxicity. Acylated derivatives, especially the *N,N*′-diacetyl DDS (DADDS), are reported to be longer acting, presumably as a consequence of slow enzymatic liberation of DDS. The dependence on enzymatic deacetylation results in erratic activity, and the rapid excretion of liberated DDS still results in low blood sulfone levels.

As part of studies aimed at determining whether a metabolite might be responsible for the antimalarial and/or the methemoglobinemia activity of DDS, we were interested in preparing 4,4′-dihydroxylaminodiphenyl sulfone. In the course of preparing this highly labile molecule for testing, a stable acetylated hydroxylamino derivative was also prepared which proved to be active against *Plasmodium berghei*.



DDS, X = $-NH_2$
DADDS, X = $-NHAc$
DHDS, X = $-NHOH$
TAHDS, X = $-N(OAc)Ac$

For the preparation of 4,4′-dihydroxylaminodiphenyl sulfone (DHDS), we investigated a procedure similar to that reported to give 2,2′-dihydroxylaminodiphenyl sulfone by catalytic reduction of the corresponding nitro compound.[9] However, attempts to reduce 4,4′-di(nitrophenyl) sulfone in this manner led only to complex mixtures of incompletely reduced labile materials. Matsukawa and coworkers[10] reported the preparation of DHDS, mp 170° dec, by reduction of the dinitro compound with Zn and aqueous EtOH. Repeated attempts to reproduce this reaction failed. Attempted preparation using other catalysts or reagents which normally reduce nitro compounds (Al and NaOH; Al-Hg and NaOH; Na_2S)[11] gave either no reaction, mixtures of high-melting solids, or the diamine.

Study of the Matsukawa procedure led to our finding that the reduction was catalyzed by traces of AcOH.† Using AcOH, coupled with a short reaction time to minimize thermally induced rearrangements, reproducibly yielded DHDS, mp 184–186° dec. This compound was found to be

†Although there are many reports of the successful application of Zn–NH_4Cl–EtOH reduction of nitro compounds to hydroxylamines, there are also many reports of frustratingly erratic behavior[11] and the significant variable has never been identified. We have found that, at least in the present reduction, the presence of a small amount of AcOH is essential. In its absence no reaction occurred; if too much was present, reduction continued all the way to the diamine. The presence of the small amount of AcOH repeatedly gave reproducible reductions.

Table I. Comparison of DDS and Derivatives Given ip as a Suspension in Saline in Tests against *P. berghei* (5 Mice/Group, 2% of Red Cells Infected)[a]

Drug	Dose, mg/kg	No. surviving 5 times control survival time per total no. tested	Mean survival time of nonsurvivors, days
Saline control			4.8
TAHDS	40		15.8
	160	5/5 (3 free of all plasmodia)	
	640	4/5 (all free of plasmodia)	6.0
Saline control			7.8, 6.0
DHDS	40		6.6, 11.0
	160		7.4
	640		9.0
Saline control			5.1
DADDS	40	3/5 (1 free of plasmodia)	20.5
	160	5/5 (3 free of plasmodia)	
Saline control			6.3
DDS	300 (max tolerated dose)	1/6	13.8

[a] 15–18 g. Charles River ICR mice infected by ip injection of 10^6 red cells from a donor having 60–80% infected cells.

Table II. Comparison of Routes of Administration of DAHDS on Antimalarial Activity in Mice Having 5–30% of Red Cells Infected with *P. berghei*

Dose	Route	No. surviving 5 times control survival time per total no. tested	Mean survival time of nonsurvivors, days
Saline, 0.5 ml	ip		5.6 (control)
Peanut oil, 0.2 ml	sc		6.0 (control)
DMSO, 0.03 ml	iv		5.0 (control)
TAHDS in peanut oil as suspension			
160 mg/kg	sc	5/10	23.0
TAHDS in DMSO in solution			
40 mg/kg	iv		5.7
160 mg/kg	iv		7.2
640 mg/kg	iv		10.2
TAHDS in water as suspension			
640 mg/kg	Oral		12.8
TAHDS in saline as suspension			
40 mg/kg	ip	1/5	17.3
160 mg/kg	ip	2/5	19.7

very sensitive to thermal and acid- or base-catalyzed rearrangements and also to undergo rearrangement on prolonged standing in EtOH. The rearrangement products were mixtures of very high-melting solids whose nature has not yet been established. Acetylation of DHDS gave the tetraacetylated O,N derivative TAHDS which proved to be easily isolated and stable to acid and alkali. This stability was surprising since diacylhydroxylamines, intermediates in the Lossen rearrangement,[12] are normally quite labile. While diformyl DDS is easily hydrolyzed to DDS at room temperature under acid conditions, and diacetyl DDS yields DDS quantitatively when boiled in 1.2 *N* HCl, TAHDS is not affectd by aqueous 6 *N* HCl at 100°.

DHDS itself had no antimalarial activity even at does as high as 640 mg/kg ip (see Table I). However, mice inoculated with *P. berghei* and treated 2.5 days after infection with TAHDS, 40 mg/kg ip, survived for 15.8 days, while controls, infected with 2% parasitized red cells, died within 5.8 days (Table I). TAHDS was "curative" (curative = treated mice survive five times the untreated control survival time) at dose levels of 160 and 640 mg/kg ip. DDS at a dose level of 300 mg/kg was curative in only one of six mice, while DADDS was curative to 3/5 mice at 40 mg/kg and 5/5 mice at 160 mg/kg. In a similar test DHDS at 600 mg/kg did not prolong the survival time of mice. In another experiment a single dose of 50 or 400 mg/kg sc (in 0.1% sodium carboxymethylcellulose) of TAHDS, given 4 hr after infection, completely protected mice from developing *P. berghei* (no parasites found in blood smears 15 days after infection) while nontreated mice died within 7–8 days. Five out of ten mice showing 5–30% parasitemia (after 2.5 days) were "cured" after treatment with TAHDS in peanut oil at 160 mg/kg sc, while the other five survived for 23 days (Table II). The untreated mice died within 5.6 days. TAHDS showed no activity against *P. berghei* when administered orally or intravenously (in DMSO) at doses as high as 640 mg/kg. These data indicated the desirability of a repository route of medication for TAHDS; this was affirmed by administering the saline suspension ip. TAHDS at 40 mg/kg was now curative to 1/5 mouse and increased survival time in the other mice to 17.3 days; 160 mg/kg was curative to 2/5, with survival 19.7 days for the remaining three (Table II).

Because DDS is known to cause methemoglobin formation (possibly through metabolism to a monohydroxylamine derivative), it was of interest to examine the effect of administering DHDS and TAHDS on methemoglobin formation. When 160 or 640 mg/kg of TAHDS was given to dogs, no methemoglobin was produced; the same doses of DHDS caused marked methemoglobinemia. TAHDS (4% in DMSO) added to human blood at a final concentration of 200 μg/ml caused a 2.3% formation of methemoglobin at room temperature, 4.2% at 37° after 30 min, and 19.7 and 19.4% after 24 hr.

In the cat TAHDS, at a dose of 160 mg/kg iv, caused the methemoglobin level to increase to 12.5% after 15 min. This implies some deacetylation to DHDS in the cat and by human blood. It should be noted that these are much higher concentrations of drug than could be attained in the absence of an organic solvent. No studies were done on methemoglobin in mice but during the malaria tests no cyanosis was apparent to the observers even at doses of 640 mg/kg iv.

The lack of antimalarial activity of DHDS in a system in which TAHDS was highly active indicates that the activity of DDS is not due to metabolic conversion to the bishydroxylamine. Neither can the antimalarial activity of TAHDS be due to deacetylation to DHDS since DHDS is inactive. In this latter respect, TAHDS differs from the other DDS derivatives which are presumed to be active because of enzymatic deacylation to DDS. The possibility remained that the activity of TAHDS might be the result of some type of metabolic conversion directly to DDS. This point was examined further.

DDS is assayed by measuring the color developed by the Bratton-Marshall reagent for aromatic amines;[13] TAHDS does not give a color with this reagent. TAHDS in DMSO, incubated with mouse plasma for 1 hr at 37°, caused formation of 10% of a positively reacting substance (calculated as DDS equivalents). After administration (60 min) to mice at a dose of 160 mg/kg iv, there was only a 10% conversion to substances giving a positive Bratton-Marshall test; this is a low degree of transformation. It has not been established that the material giving the positive response to the Bratton-

Marshall reagent is DDS. Calculations based on other studies[14] have shown that blood levels of 2-3 μg/ml of DDS are required to prevent the development of *P. berghei* infection in mice. TAHDS showed significant antimalarial activity at 40 mg/kg ip; based on the methemoglobin studies, a maximum of 10% of that absorbed might be present as DDS. It seems unlikely that levels of 2-3 μg/ml would be achieved after administration of 40 mg/kg of a compound of such poor solubility and low possible DDS yield *in vivo*.

TAHDS was tested against two other parasites. It showed little activity against *Trichinella spiralis* in mice (at 25 mg/kg orally plus 25 mg/kg sc) or against *Eimeria tenella* in chicks fed diets containing 0.05% TAHDS for 2 days prior to and 6 days after infection.

In mice, TAHDS showed antileprotic activity against *Mycobacterium leprae* approaching that of DDS. It had no activity against a variety of tumors.

TAHDS produced no overt effects or signs of toxicity in any of the mouse tests or when administered orally to rats at a dose of 200 mg/kg. It did not exacerbate adjuvant-induced arthritis in rats or inhibit gastric acid secretion in gastric fistula rats at a dose of 50 mg/kg po; 24 hr after administration of 80 mg/kg po to chronic, metacorticoid-hypertensive rats, there was a significant reduction in systolic blood pressure.

Experimental Section‡

4,4′-Dihydroxylaminodiphenyl Sulfone (DHDS). Bis(*p*-nitrophenyl) sulfone[15] (54 g, 0.18 mol) was suspended in 1.2 l. of 80% EtOH containing several milliliters of AcOH. Ammonium chloride (90 g) and Zn dust (65 g) were added and the mixture was heated at reflux with stirring for 45 min. The resulting pale yellow solution was filtered, the filtrate was concentrated to 150 ml, and 700 ml of H_2O preheated to 95° was added to this warm solution. A small amount of orange gum formed after 2 or 3 min and the mixture was then immediately filtered. After 5 min, a crystalline solid separated from the filtrate. The solution was rapidly chilled and filtered, giving 32 g of light yellow needles, mp 160–165° (170° dec). This crude DHDS was suitable for use in the acetylation reaction. DHDS is unstable in aqueous solution, being converted to a very high-melting (greater than 300°) mixture of solids. Further purification is tedious and difficult to reproduce, but 1-2-g amounts could be purified as follows. The crude hydroxylamine was dissolved in a minute quantity of preheated (95°) H_2O and filtered by suction 3–4 times as the solution slowly cooled. When the first crystals started to form, the solution was rapidly chilled and the crystalline solid was immediately filtered. After three recrystallizations, the crystals were obtained as colorless needles, mp 184–186° dec (lit.[10] mp 170° dec). *Anal.* ($C_{12}H_{12}N_2O_4S$) C, H, S. When this material is slowly heated, left in aqueous solution for a lengthy period, or treated with acids or bases, it is converted to a mixture of high-melting solids whose nature has not been determined.

4,4′-Bis(*N,O*-diacetylhydroxylamino)diphenyl Sulfone (TAHDS). A suspension of 30 g of crude DHDS in 400 ml of Ac_2O was heated on a steam bath. After 15 min all solids dissolved giving a clear orange solution. Excess Ac_2O was removed *in vacuo*, EtOH was added to the residue, and the suspension was again concentrated *in vacuo* giving an orange slush. This was triturated with 150 ml of EtOH; then 600 ml of H_2O was added and the resulting gummy solid was filtered (60 g), mp 167–172°. The gum was triturated with $CHCl_3$ and filtered. The insoluble solid, 3 g, mp 260–265°, was discarded (probably DADS). The filtrate was dried ($MgSO_4$) and then concentrated to give 34 g of orange oil that crystallized on cooling. This was recrystallized from EtOH and then three times from $CHCl_3$–hexane to give 13 g of white solid, mp 186–188°. Concentration of the filtrate gave another 10 g, mp 179–183°. *Anal.* ($C_{20}H_{20}N_2O_8S$) C, H, N, S. The solubility of TAHDS is low (<2.5 γ/ml in H_2O, <100 γ/ml in EtOH); however, 120 mg/ml may be dissolved in DMSO. In the search for a convenient assay for THDS that could be used with plasma, TAHDS was heated with Zn and HCl at 100°. The products did not contain DDS (as shown by tlc) nor did they give a positive color reaction for arylamines with the Bratton–Marshall reagent.[13]

‡Melting points are corrected; boiling points are uncorrected. Elemental analyses were performed by Miss M. Carroll and coworkers of the Analytical and Physical Chemistry Section, Smith Kline & French Laboratories. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

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L-4′-Cyano-3-(2,2,2-trifluoroacetamido)succinanilic Acid and Related Synthetic Sweetening Agents

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Since the discovery of saccharin in 1879[1] a number of sweetening agents have been described.[2-5] These compounds are all of interest, since they reflect the diversity of chemical structure and the complete lack of any unifying generalization that would aid in predicting sweetness.

In 1968 the pronounced sucrose-like taste of L-aspartyl-L-phenylalanine methyl ester (**43**) was reported.[6] Although no other compound more patently sweet than the initial discovery was found, the retention of sweetness was noted to be correlated with both the unsubstituted amino and β-carboxy groups of aspartic acid. L-Aspartic acid could not be replaced without loss of sweetening activity, but con-