activity of both acetylcholine and carbachol. The dose–response curves of acetylcholine (10^{-7} *M*) antagonized by **1** or **2** were identical within experimental error. The ED_{50} for both **1** and **2** was 0.07 mg/ml (*ca.* 4×10^{-4} *M*).

Previous workers have studied acetylcarbocholine[4] and acetylsilicocholine[5] and have concluded that these two compounds act as indirect, nonnicotinic, cholinergic agents. This study implies that both **1** and **2** have an affinity for the muscarinic site but do not possess intrinsic activity.

Experimental Section

The procedure described by Turner[6] was followed for bioassay purposes. The bath temperature was maintained at 37 ± 1°, and oxygenated Tyrode's solution was used as the perfusing fluid. Hexamethonium bromide (10 mg/l.) was added to the Tyrode's solution to prevent ganglionic response. The final bath volume for each run was 30 ml and chemical concentrations were calculated accordingly.

The test compounds could only be dissolved in Tyrode's solution with the aid of ethanol. Delivery of 1 ml of the test solution to 29 ml of bath gave a final concentration of 1.6% ethanol. This did not have a significant influence on the muscle response induced by 10^{-7} *M* acetylcholine.

A typical run was carried out in the following manner. The ileum was dissected, then washed with Tyrode's solution. Approximately 5 cm of the muscle was attached to a transducer in the muscle bath (29 ml) and allowed to stand until the spontaneous contractions had subsided. The transducer was connected to a strip chart recorder. Acetylcholine chloride solution (1 ml) (final bath concentration 10^{-7} *M*, 1.6% ethanol) was delivered to the bath. The amplitude of the strip chart recorder was adjusted so that the resulting contraction gave a 70–90% pen deflection. The muscle was then washed continuously with Tyrode's solution until the muscle had relaxed. The wash solution was drained, 29 ml of fresh Tyrode's solution was added to the bath, and the system was allowed to equilibrate. After the pen deflection was adjusted, the calibration was repeated at least five times during and at the end of the assays of the test compounds. The average of these values was taken as the 100% contraction value for 10^{-7} *M* acetylcholine for the particular muscle.

The test compounds were assayed in essentially the same manner. The initial bath volume was 28 ml. To this was added 1.0 ml of the test solution followed within about 15 sec by 1.0 ml of the acetylcholine solution (final bath concentration, 10^{-7} *M* ACh). The process was repeated three times and the average of the pen deflections was taken as the value for that particular concentration of test compound. This value was then expressed as a per cent of the contraction value for 10^{-7} *M* ACh. At least four animals were used for each compound. The data that were obtained are summarized in Figure 1.

The test compounds alone gave no muscle response in the dose range of 0.30 to 2.5×10^{-5} mg/ml.

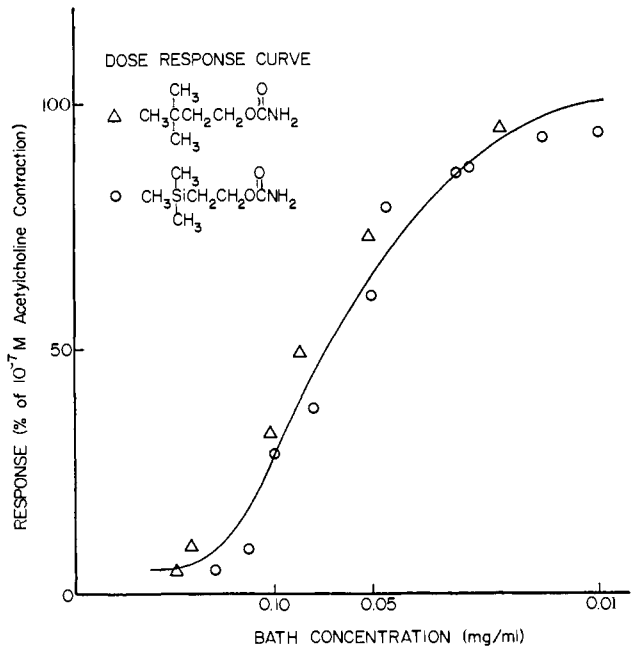

Figure 1.

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(6) R. A. Turner, "Screening Methods in Pharmacology," Academic Press Inc., New York, N. Y., 1965, pp 42–46.

Potential Antimalarial Substances. Antimetabolites of Pantothenic Acid[1]

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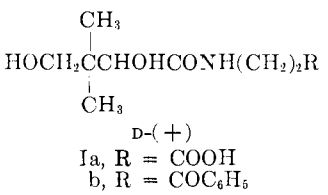
In 1943 it was observed that the survival of the erythrocytic stages of *Plasmodium lophurae*, maintained intracellularly in duck red cell suspensions *in vitro*, was favored by the addition of calcium pantothenate to the medium.[2] Moreover, the development of *Plasmodium gallinaceum* in chickens is inhibited by a deficiency in pantothenic acid (Ia).[3] These observations stimulated a search for antimetabolites that might interfere with the utilization of pantothenic acid by plasmodia.[3–7] Among the antipantothenates that were synthesized and tested earlier,[3–7] D-(+)-phenylpantothenone (Ib) was equiactive with quinine against *P. gallinaceum* and twice as potent as quinine against *P. lophurae* in the chick.[5,7] In man, Ib exhibited slight activity against blood-induced vivax malaria and was tolerated well in doses of 2 g daily for 4 days.[5] The pantoyltaurine derivatives VIa and VIb were the most active compounds synthesized; both were approximately ten times as potent as quinine against *P. gallinaceum* in chicks.[5,6]



More recently it was shown that pantothenic acid has no effect on the erythrocytic stages of *P. lophurae*

(1) This investigation was supported by U. S. Army Medical Research and Development Command Contract DA-49-193-MD-2754. This is Contribution No. 360 to the Army Research Program on Malaria.

(2) W. Trager, *J. Exp. Med.*, **77**, 411 (1943).

(3) S. Brackett, E. Waletzky, and M. Baker, *J. Parasitol.*, **32**, 453 (1945).

(4) J. F. Mead, M. M. Rapport, A. E. Senear, J. T. Maynard, and J. B. Koepfli, *J. Biol. Chem.*, **163**, 465 (1946).

(5) F. Y. Wiselogle, "A Survey of Antimalarial Drugs, 1941–1945," Vol. I, J. T. Edwards, Ann Arbor, Mich., 1946, pp 138–140.

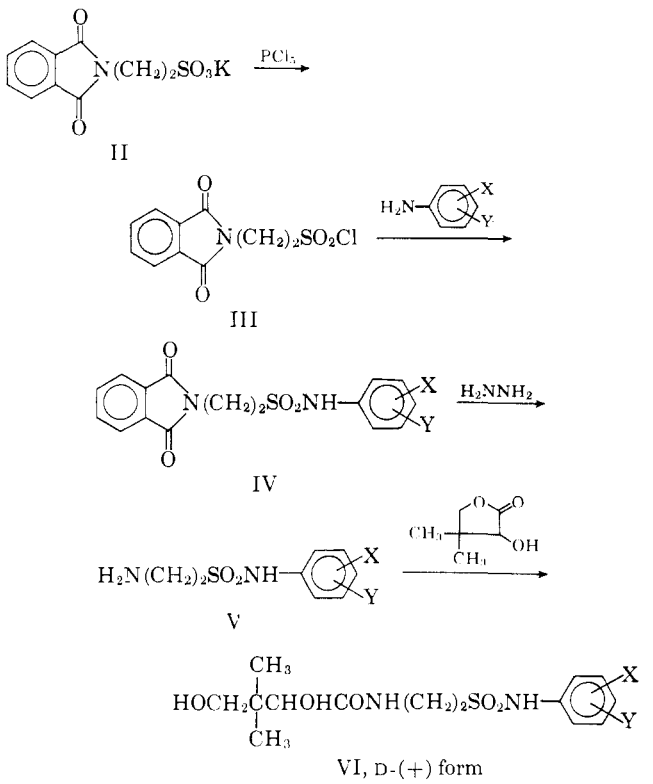
(6) R. Winterbottom, J. W. Clapp, W. H. Miller, J. P. English, and R. O. Roblin, Jr., *J. Am. Chem. Soc.*, **69**, 1393 (1947).

(7) R. E. Lutz, J. W. Wilson, III, A. J. Deinet, G. H. Harnest, T. A. Martin, and J. A. Freek, *J. Org. Chem.*, **12**, 96 (1947).

removed from their host red cells and maintained extracellularly *in vitro;* their survival under these conditions is favored by coenzyme A.[8,9] Therefore, it was concluded that malaria parasites require preformed CoA supplied to them by the host erythrocyte. Moreover, the antipantothenate VIb inhibits *P. lophurae* when it is developing intracellularly, but not when it is cultured extracellularly *in vitro.*[10] The antipantothenate VIb presumably interferes with a red cell system synthesizing CoA, thereby depriving the parasite of an adequate source of CoA. It likewise appears that CoA is a limiting factor for the monkey parasite *Plasmodium coatneyi* and the human parasite *Plasmodium falciparum* since antipantothenates also inhibit the intraerythrocytic development of these plasmodia.[10]

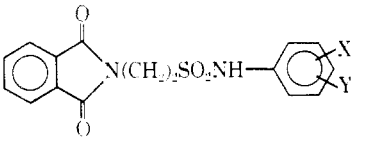
To enable further evaluation of the potential usefulness of the antipantothenates in malaria chemotherapy, the preparation of an authentic sample of VIb and related D-(+)-2,4-dihydroxy-3,3-dimethyl-N-[2-(phenylsulfamoyl)ethyl]butyramides (VI) (Table III) was initiated. The compounds were synthesized according to Scheme I.[6,11] The experimental procedures outlined previously[6,11] were generally satisfactory. However, the hydrazinolysis of IVd (X, Y = H, 3-CF_3) (Table I) gave a poor yield of 2-amino-3′-(trifluoromethyl)ethanesulfonanilide (Vd) (Table II) and the sequence was not completed. Further, the condensation of Vf [X, Y = 3,5-$(CF_3)_2$] with D-(−)-pantolactone gave a mixture from which Vf was not isolated in pure form.

SCHEME I



a, X, Y = H
b, X, Y = 4-Cl
c, X, Y = 3,4-Cl_2
d, X, Y = 3-CF_3
e, X, Y = 4-CF_3
f, X, Y = 3,5-$(CF_3)_2$
g, X, Y = 4-SCH_3
h, X, Y = 4-$OCH_2C_6H_5$

TABLE I
1,3-DIOXO-2-ISOINDOLINEETHANESULFONANILIDES (IV)[a]



No.	X, Y	Mp, °C	Yield (purified), %	Formula
IVb	4-Cl	143–147[b]	61	$C_{16}H_{13}ClN_2O_4S$
IVc	3,4-Cl_2	173–174	64	$C_{16}H_{12}Cl_2N_2O_4S$
IVd	3-CF_3	128–130	79	$C_{17}H_{13}F_3N_2O_4S$
IVe	4-CF_3	168–171	85	$C_{17}H_{13}F_3N_2O_4S$
IVf	3,5-$(CF_3)_2$	154–159	98	$C_{18}H_{12}F_6N_2O_4S$
IVg	4-SCH_3	152–155	52	$C_{17}H_{16}N_2O_4S_2$
IVh	4-$OCH_2C_6H_5$	155–158	54	$C_{23}H_{20}N_2O_5S$

[a] These intermediates were demonstrated to be homogeneous by thin layer chromatography, and were not subjected to microanalysis but were used directly in the next step. [b] Lit.[6] mp 154–155°.

The D-(+)-2,4-dihydroxy-3,3-dimethyl-N-[2-(phenylsulfamoyl)ethyl]butyramides (VIb, c, e, g, h, Table III) and the 2-aminoethanesulfonanilides Vf and h (Table II) were administered subcutaneously in a single dose to mice infected with *P. berghei.*[12,13] None of these compounds, including VIb, caused a significant prolongation of the mean survival time of mice even at the highest dose level employed, namely 640 mg/kg. Further, when representative compounds (VIb, VIc, and VIg) were given continuously in the diet for 6 days to mice infected with another strain of *P. berghei* in daily doses ranging from 311 to 366 mg/kg, no significant reduction in parasitemia was noted among the treatment groups.[14]

The antipantothenates can thus be added to the growing list of substances that reportedly are effective against avian malarias, but which lack appreciable effects on *P. berghei* in mice: 5-phenyl-2,4-pentadienamides,[15] 3-amino-7-chloro-1,2,4-benzotriazines,[16] glyoxal bis(thiosemicarbazones),[16] and 3-amino-2,6-dichloroquinoxalines.[17] Studies are planned to confirm the reported activity of the antipantothenates against other malarias, and a satisfactory explanation is being sought for the apparent discrepancy between earlier reports[3-7] and results of the current investigation utilizing *P. berghei.*

Representative compounds were also tested against other microorganisms *in vitro,* including *Trichomonas vaginalis, Staphylococcus aureus* (UC-76), *Pseudomonas aeruginosa* (No 28), *Mycobacterium tuberculosis* ($H_{37}Rv$).

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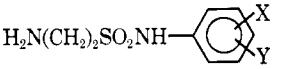
(9) W. Trager, *J. Protozool.*, **1**, 231 (1954).
(10) W. Trager, *Trans. N. Y. Acad. Sci.*, **28**, 1094 (1966).
(11) W. H. Miller and R. O. Roblin, Jr., U. S. Patent 2,459,111 (1949).
(12) The initial antimalarial screening was carried out by Dr. Leo Rane of the University of Miami, and test results were supplied through the courtesy of Dr. David P. Jacobus of the Walter Reed Army Institute of Research.
(13) For a description of the test method, see T. S. Osdene, P. B. Russell, and L. Rane, *J. Med. Chem.*, **10**, 431 (1967).
(14) Selected compounds were kindly evaluated by drug-diet against *P. berghei* in mice by Dr. Paul E. Thompson and co-workers, Research Laboratories, Parke, Davis and Co., Ann Arbor, Mich.
(15) L. M. Werbel, N. Headen, and E. F. Elslager, *J. Med. Chem.*, **10**, 366 (1967).
(16) E. F. Elslager, presented at the 153rd National Meeting of the American Chemical Society, Miami Beach, Fla., April 10, 1967.
(17) E. F. Elslager, C. A. Hess, and L. M. Werbel, *J. Med. Chem.*, **11**, 630 (1968).

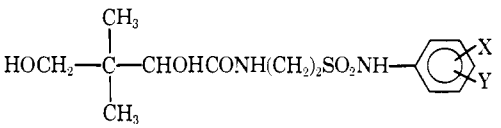
TABLE II
2-AMINOETHANESULFONANILIDES (V)



No.	X, Y	Mp, °C	Yield purified, %	Purifn solvent	Formula	Analyses[19]
Vb	4-Cl	160–162[a]	66	...	$C_8H_{11}ClN_2O_2S$	*b*
Vc	3,4-Cl_2	157–159	64	...	$C_8H_{10}Cl_2N_2O_2S$	*b*
Vd	3-CF_3	154–156	3	EtOAc	$C_9H_{11}F_3N_2O_2S$	C, H, N
Ve	4-CF_3	163–167	76	...	$C_9H_{11}F_3N_2O_2S$	C, H, N
Vf	3,5-$(CF_3)_2$	262–263 dec	44	*i*-PrOH	$C_{10}H_{10}F_6N_2O_2S \cdot HCl$	C, H, N, Cl
Vg	4-SCH_3	162–165	75	H_2O	$C_9H_{14}N_2O_2S_2$	*b*
Vh	4-$OCH_2C_6H_5$	211–214	94	H_2O	$C_{15}H_{18}N_2O_3S \cdot HCl$[c]	C, H, N

[a] Lit.[6] mp 160.5–161.5°. [b] These intermediates were not analyzed. They were homogeneous by tlc and were used directly in the next step. [c] Free base, mp 178–181°.

TABLE III
D-(+)-2,4-DIHYDROXY-3,3-DIMETHYL-N-[2-(PHENYLSULFAMOYL)ETHYL]BUTYRAMIDES (VI)



No.	X, Y	Mp, °C	Yield purified, %	Purifn solvent	$[\alpha]^{25}D$,[a] deg	Formula[c]
VIb	4-Cl	103–104[b]	59	C_6H_6	+40	$C_{14}H_{21}ClN_2O_5S$
VIc	3,4-Cl_2	141–143	66	$CHCl_3$	+38	$C_{14}H_{20}Cl_2N_2O_5S$
VIe	4-CF_3	144–146	45	H_2O	+36	$C_{15}H_{21}F_3N_2O_5S$
VIg	4-SCH_3	112–114	67	$ClCH_2CH_2Cl$	+40	$C_{15}H_{24}N_2O_5S_2$
VIh	4-$OCH_2C_6H_5$	142–144	28	$ClCH_2CH_2Cl$	+33	$C_{21}H_{28}N_2O_6S$

[a] *c* 1, 95% EtOH. [b] Lit.[6] mp 101–103° from C_6H_6. [c] All compounds were analyzed for C, H, N.

Escherichia coli (Vogel), *Streptococcus pyogenes* (C203), *Proteus mirabilis* (MGH-1), *Salmonella typhimurium* (V-31), and *Shigella sonnei* (C-10). Among them, VIh suppressed *T. vaginalis in vitro* at a concentration of 25 μg/ml and completely inhibited the growth of *S. pyogenes* (C203) at 1.25 μg/ml.

Experimental Section[18,19]

1,3-Dioxo-2-isoindolineethanesulfonic acid monopotassium salt (II) was prepared by the method of Miller and Roblin[6,11] in 83% yield.

1,3-Dioxo-2-isoindolineethanesulfonyl chloride (III) was obtained from II by the method of Miller and Roblin[6,11] in 80% yield, mp 158–162°.

1,3-Dioxo-2-isoindolineethanesulfonanilides (IV, Table I).—To a stirred solution of 0.1 mole of the substituted aniline in 75 ml of pyridine, cooled with an ice bath, was added slowly 30.1 g (0.11 mole) of 1,3-dioxo-2-isoindolineethanesulfonyl chloride (III). After the reaction mixture was stirred for 1 hr with cooling, the ice bath was removed and stirring was continued for 1.25 hr. The reaction mixture was poured into 500 ml of H_2O with vigorous stirring, and the crude product was isolated by filtration. Recrystallization from glacial or dilute AcOH gave the product.

2-Aminoethanesulfonanilides (V, Table II).—A mixture of 0.02 mole of the appropriate 1,3-dioxo-2-isoindolineethanesulfonanilide, 1.2 g (0.02 mole) of 85% hydrazine hydrate, and 100 ml of EtOH was heated under reflux for 3 hr. The reaction solution was homogeneous when refluxing began, but after 15 min a precipitate appeared. The mixture was concentrated to dryness and the residue was suspended in 200 ml of H_2O and made acidic to congo red with 4 *N* HCl. This slurry was heated on a steam bath for 10 min, cooled in an ice bath, and filtered. The filtrate was neutralized with concentrated NH_4OH to give the product. The compounds were recrystallized from the indicated solvents when necessary.

(18) Melting points (corrected) were taken in open capillary tubes in a Thomas–Hoover capillary melting point apparatus.

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

D-(+)-2,4-Dihydroxy-3,3-dimethyl-N-[2-(phenylsulfamoyl)-ethyl]butyramides (VI, Table III).—A mixture of 0.015 mole of the requisite 2-aminoethanesulfonanilide (V) and 3.9 g (0.03 mole) of D-(−)-pantolactone was heated in a melt at 100–115° for 2 hr. The melt was cooled and crystallized from the solvents indicated.

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Derivatives of 5-Phenyl-2,4-pentadienoic Acid as Potential Antimalarial Agents[1]

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The reported effectiveness of 5-(*p*-chlorophenyl)-*N*-isopropyl-2,4-pentadienamide (Ia) against *Plasmodium gallinaceum* in the chick[2] prompted the synthesis of

(1) This investigation was supported by the U. S. Army Medical Research and Development Command under Contract DA-49-193-MD-2754. This is Communication No. 382 to the Army Research Program on Malaria.

(2) G. R. Coatney, W. C. Cooper, N. B. Eddy, and J. Greenberg, "Survey of Antimalarial Agents," Public Health Service Publication No. 193, Washington, D. C., 1953, pp 98, 139, 262, 276.