THE SYNTHESIS OF POTENTIAL ANTIMALARIALS

DERIVATIVES OF PANTOYLTAURINE*

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The general hypothesis as to the mode of action of chemotherapeutic agents, which has been formulated by Fildes, Woods, McIlwain, and others (2), offers a rational and useful guide to the design of new drugs. Thus, bacteriostasis is pictured as caused by the blocking of reactions essential to growth by an inhibiting substance which has a structure similar to that of one of the normal enzymes or metabolites essential to the growth of the organism.

There is some indication that such a mechanism may also be involved in the case of protozoa, since it is known that certain of the sulfonamides are plasmodicidal *in vivo*. To extend this approach to *Plasmodium* by attempting to block essential metabolites or enzymes other than those involved in the action of the sulfonamides has been made difficult by the lack of knowledge of the essential metabolic requirements of the parasite.

An important lead to this approach has been furnished by Trager (3), who showed that the survival *in vitro* of *Plasmodium lophurae* is favored by the presence of calcium pantothenate. Although the evidence is indirect, Trager's results indicate that pantothenic acid is the only growth factor of known chemical structure thus far demonstrated for any species of *Plasmodium*.

The hypothesis of Fildes *et al.* has been tested experimentally by the design and preparation of several new growth inhibitors for bacteria (2). In the case of pantothenic acid, Snell (4) was the first to report the preparation of a salt of *dl*-pantoyltaurine which inhibited the growth of certain bacteria *in vitro* and he showed that the inhibition was reversed by panto-

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The simplified nomenclature has been employed (see Barnett and Robinson (1)) in which "pantoyl" is used for the α,γ-dihydroxy-β,β-dimethylbutyryl radical. The designation "*d*" and "*l*" has been used to indicate only that the compound is dextrorotatory or levorotatory.

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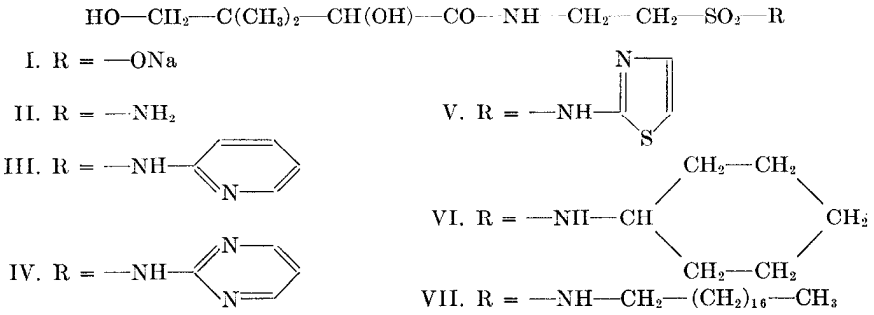
thenic acid. In the same year Kuhn, Wieland, and Möller (5) prepared solutions of the *d* and *l* modifications of pantoyltaurine and they reported that the *d* form was 32 times more active than the *l* form as a growth inhibitor for certain bacteria. Independently, McIlwain, Barnett, and Robinson (6) prepared and tested as bacterial inhibitors not only *dl*-pantoyltaurine but also *dl*-pantoyltaurylamide and a number of other analogues of pantothenic acid.

In the light of Trager's evidence that pantothenic acid is an essential growth factor for *Plasmodium lophurae* and of the work of Snell, McIlwain, and Kuhn, showing the existence of several compounds known to interfere with pantothenic acid metabolism, at least with respect to bacteria, the advisability of testing pantothenic acid inhibitors for antimalarial activity becomes evident.

The work reported here describes the preparation, in as pure a form as possible, of the optically active *d*-pantoyltaurine (I) and several of its derivatives, II, III, IV, V, VI, and VII, primarily for testing as antimalarials and incidentally for testing with a variety of other pathogens.



A report of the results obtained with compounds I to VII in tests on avian malaria will be reported elsewhere.[1] It may be stated, however, that *d*-pantoyltaurylamide (II) showed definite activity *in vivo* in the suppression of parasites under certain test conditions.

EXPERIMENTAL

d-Pantoyltaurine (Sodium Salt) (I), (SN 327)[1]—This compound was prepared in a manner similar to that employed by Snell (4) for the preparation of the optically inactive compound. The preparation of solutions of the *d* and *l* modifications has been reported by Kuhn *et al.* (5).

To 36.4 gm. of thoroughly dried *l*-pantolactone were added 41.2 gm. of

[1] The Survey number, designated SN, identifies a drug in the records of the Survey of Antimalarial Drugs. The antimalarial activities of the compounds to which Survey numbers have been assigned will be tabulated in a forthcoming monograph.

the dried sodium salt of taurine and the mixture heated between 115–120° for 4.5 hours with occasional stirring. Bubbles of gas appeared to evolve during the reaction. The melt was poured into absolute ethanol and, upon standing, formed a gel. The product was extracted with 700 ml. of absolute ethanol, the ethanol concentrated *in vacuo* to 100 ml., and 600 ml. of acetone added. A powder precipitated which, after standing overnight at 0°, was filtered, washed with acetone, and dried over sulfuric acid *in vacuo*. There were obtained 46.5 gm. (90 per cent on the basis of lactone consumed) of a white deliquescent powder melting with effervescence at 100–110°. $[\alpha]_D^{23} = +23.4°$ (27.2 mg. in 1.93 ml. of water).

$C_8H_{16}O_6NSNa$.	Calculated.	C 34.7, H 5.8, N 5.1, Na 8.3
	Found.	" 34.5, " 6.1, " 4.8, " 8.4

d-Pantoyltaurylamide (II), (SN 3279)—Taurylamide hydrochloride, prepared by the method of Miller, Sprague, Kissinger, and McBurney (7), was converted to the free base as described by Barnett and Robinson (8). The oil thus obtained crystallized to a solid melting at 90–100°; because it is hygroscopic, it was used without further purification. Taurylamide (19.3 gm.) was heated with *l*-pantolactone (21.0 gm.) at 100–110° for 3 hours (see Barnett and Robinson's (8) procedure for the preparation of the optically inactive compound). The product (39 gm.) was a hygroscopic viscous gum which could not be induced to crystallize. A sample was purified for analysis by repeated precipitations from ethanol with isopropyl ether; after prolonged drying over phosphorus pentoxide *in vacuo*, the *d*-pantoyltaurylamide consisted of a colorless glassy solid. $[\alpha]_D^{23} = +19.1°$ (52 mg. in 2.0 ml. of water).

$C_8H_{18}O_5N_2S$. Calculated, N 11.0; found,[2] N 10.7

β-Phthalimidoethanesulfonyl-2-aminopyridine—Since the condensation of the acid chloride and 2-aminopyridine, whether carried out in pyridine or in water with sodium carbonate, always resulted in the hydrolysis of the sulfonyl chloride, the following conditions were employed: To 25 gm. of 2-aminopyridine in the minimum amount of benzene were added 35 gm. of β-phthalimidoethanesulfonyl chloride (7) in benzene. The resulting solution was refluxed for 1 hour and allowed to cool. The precipitate, which formed during the reaction, was filtered off, stirred with dilute sodium bicarbonate solution, filtered, washed with water, and dried over sulfuric acid *in vacuo*. For purification, this product was refluxed with 150 ml. of methanol, allowed to cool, and filtered. There were thus obtained 33 gm. of β-phthalimidoethanesulfonyl-2-aminopyridine, colorless prisms, m.p. 213–215°.

[2] A semimicro-Kjeldahl determination was carried out by Mr. C. T. Redemann.

$C_{15}H_{13}O_4N_3S$.	Calculated.	C 54.4, H 4.0, N 12.7
	Found.	" 54.7, " 4.0, " 12.4

Tauryl-2-aminopyridine—To 23 gm. of the above β-phthalimidoethanesulfonyl-2-aminopyridine suspended in 200 ml. of hot ethanol were added 11.7 ml. of 42 per cent hydrazine hydrate (9), and the mixture was refluxed on the water bath. During 15 minutes, the solid had dissolved, and a precipitate had begun to form. The mixture was refluxed for 1 hour, cooled, and filtered. The intermediate thus obtained was dissolved in 1 liter of hot water and treated with 13.5 ml. of concentrated hydrochloric acid. When the solution had cooled, the phthalhydrazide was filtered off, and the filtrate evaporated to dryness. The solid thus obtained, after several crystallizations from ethanol, proved to be a mixture of the mono- and dihydrochlorides of taurylaminopyridine. Although they could never be completely separated, it was found that the monohydrochloride melted at about 165°, while the dihydrochloride had a melting point of about 190°. The hydrochlorides were each treated with the required amount of sodium bicarbonate solution. On evaporation of the aqueous solutions and crystallization of the residues from absolute ethanol, the same compound was obtained from both hydrochlorides as colorless clusters of platelets, m.p. 140–141°.

$C_7H_{11}O_2N_3S$.	Calculated.	C 41.8, H 5.5, N 20.9
	Found.	" 41.9, " 5.8, " 20.8

d-N²-(Pantoyltauryl)-2-aminopyridine (III), (SN 3280)—To 4.5 gm. of tauryl-2-aminopyridine were added 3 gm. of *l*-pantolactone, and the mixture was heated at 115–120° for 5 hours. After standing overnight in a desiccator over sulfuric acid, a product was obtained which solidified upon dissolving in acetone and pouring the solution into a large volume of dry ether. The material which precipitated was filtered under anhydrous conditions and dried over phosphorus pentoxide *in vacuo*. The 5.5 gm. of semicrystalline material thus obtained were hygroscopic and gradually became dark and gummy on exposure in the air. The best samples decomposed so rapidly that satisfactory analyses were not obtained. They had a melting point of about 53°. $[\alpha]_D^{23} = +18.5°$ (62 mg. in 2.0 ml. of water).

β-Phthalimidoethanesulfonyl-2-aminopyrimidine—To 17.2 gm. of 2-aminopyrimidine suspended in 50 ml. of dry pyridine were added slowly, with shaking, 46.5 gm. of β-phthalimidoethanesulfonyl chloride. The solid gradually dissolved, and, after an hour, a flocculent precipitate began to form. The suspension was stirred vigorously overnight and then poured into 1 liter of water. The resulting suspension was neutralized with sodium bicarbonate and filtered. After drying, the product was obtained as 34.3 gm. (58 per cent) of a brown powder melting at 245–250°. After several

recrystallizations from glacial acetic acid a sample was obtained as colorless prisms, m.p. 245–247°.

$C_{14}H_{12}N_4O_4S$. Calculated, C 50.6, H 3.6; found, C 50.5, H 3.7

Tauryl-2-aminopyrimidine Hydrochloride—The hydrolysis of the phthalimido compound was carried out with hydrazine hydrate and hydrochloric acid solution, as described for the preparation of tauryl-2-aminopyridine. The hydrochloride was obtained in 75 per cent yield as colorless prisms from dilute ethanol, m.p. 215–216°.

$C_6H_{11}N_4O_2SCl$.	Calculated.	C 30.2, H 4.6, N 23.5
	Found.	" 30.2, " 4.6, " 23.9

Tauryl-2-aminopyrimidine Hydrate—On neutralization of an aqueous solution of the hydrochloride with sodium bicarbonate, a precipitate was obtained which after filtering and drying consisted of flat hexagonal prisms. A sample of this material, when heated, softened above 140° and melted at 151° with decomposition.

$C_6H_{10}N_4O_2S \cdot H_2O$.	Calculated.	C 32.7, H 5.5, N 25.4
	Found.	" 33.0, " 5.2, " 25.3

Tauryl-2-aminopyrimidine—On heating at 100°, the hydrate slowly decomposed. About 85 per cent of the theoretical amount of water was removed by drying at 106–110° for 4 hours in a vacuum over phosphorus pentoxide. The compound thus obtained regained the water on standing in the air. It has not been obtained in pure form because of its instability, and the crude material was used for the next reaction.

d-N²-(Pantoyltauryl)-2-aminopyrimidine (IV), (SN 7293)—Finely powdered tauryl-2-aminopyrimidine hydrate (1.1 gm.) was dried at 92° under a high vacuum for 18 hours. To the resulting solid was added 0.9 gm. of *l*-pantolactone, and the mixture heated at 97–98° for 6 hours and at 115° for an additional 30 minutes. A clear melt was obtained. The temperature was lowered to 100° and the heating continued for 8 hours. During this latter period, some crystallization was observed. The reaction mixture was dissolved in hot ethanol, filtered, and allowed to stand overnight. The supernatant solution was decanted from a small amount of hygroscopic amorphous solid and treated with isopropyl ether. After standing for some time, small clumps of crystals were deposited. After filtering and drying, the product (0.45 gm.) was recrystallized from absolute ethanol and colorless clusters of crystals, m.p. 177–178.5°, were obtained. $[\alpha]_D^{23} = +23.6°$ (21.9 mg. in 1.99 ml. of water).

$C_{12}H_{20}O_5N_4S$.	Calculated.	C 43.4, H 6.1, N 16.9
	Found.	" 43.7, " 6.1, " 16.9

β-Phthalimidoethanesulfonyl-2-aminothiazole—To 19 gm. of 2-aminothiazole in the minimum amount of benzene were added 25 gm. of the β-phthalimidoethanesulfonyl chloride in benzene, and the solution was refluxed for 1 hour. After cooling, the solid which had precipitated was filtered off and allowed to stand overnight in sodium bicarbonate solution. It was then filtered, washed with water, dried over sulfuric acid, and crystallized from glacial acetic acid. There were obtained 27 gm. of a product with a melting point of 227–228°.

$C_{13}H_{11}O_4N_3S_2$. Calculated, C 46.3, H 3.3; found, C 46.6, H 3.3

The attempted hydrolysis of this compound with hydrazine hydrate and dilute hydrochloric acid was accompanied by a large evolution of hydrogen sulfide and led to the production of a dark oil, from which no identifiable compound could be obtained.

Tauryl-2-aminothiazole Hydrochloride—A solution of 76 gm. of β-phthalimidoethanesulfonyl-2-aminothiazole in 1500 ml. of 92 per cent ethanol containing 54 gm. of sodium hydroxide was heated at 60–65° for 24 hours. It was acidified with 10 N ethanolic hydrogen chloride and, after standing in the cold for several hours, filtered from the sodium chloride. The filtrate was evaporated to dryness under reduced pressure, and the residue dissolved in acetone and filtered to remove the unchanged starting material. The oil thus obtained was extracted with 170 ml. of water in portions and decanted from a small amount of insoluble oil (starting material and phthalic acid). Evaporation of the solution gave 15 gm. of crude tauryl-2-aminothiazole hydrochloride, which was recrystallized from glacial acetic acid to give 13.3 gm. of rosettes of needles, m.p. 187–190.5°. An analytical sample melted at 193–195°. From the reaction there were recovered 29 gm. of starting material.

$C_5H_{10}O_2N_3S_2Cl$.	Calculated.	C 24.6, H 4.1, N 17.2
	Found.	" 24.8, " 3.8, " 17.2

N^2-(Pantoyltauryl)-2-aminothiazole (*V*), (*SN 9667*)—To 1.7 gm. of tauryl-2-aminothiazole hydrochloride in 2 ml. of warm water was added 0.6 gm. of sodium bicarbonate. The resulting brown solution was dried *in vacuo* over sulfuric acid and finally over phosphorus pentoxide. To the above mixture of tauryl-2-aminothiazole and sodium chloride was added 0.91 gm. of *l*-pantolactone, and the mixture heated at 80–90° for 2 hours. The product was extracted with 10 ml. of hot absolute ethanol, filtered, and the solvent removed under reduced pressure. The residual brown oil was washed repeatedly with cold acetone and finally dried *in vacuo* over phosphorus pentoxide, whereupon it solidified to a light brown powder. The product was soluble in water, but, on standing in solution, decomposed with evolu-

tion of hydrogen sulfide. It was also unstable in moist air, but remained unchanged over long periods when kept over phosphorus pentoxide. No significant rotation could be observed.

$C_{11}H_{19}O_5N_3S_2$.	Calculated.	C 39.2, H 5.7, N 12.5
	Found.	" 39.5, " 5.8, " 12.8

β-Phthalimidoethanesulfonylaminocyclohexane—To 13.7 gm. of β-phthalimidoethanesulfonyl chloride dissolved in 250 ml. of acetone were added 10.5 gm. of cyclohexylamine. The solution was refluxed for 1 hour and then poured into a large volume of water. The solid was recovered by filtration and recrystallized from ethanol to give 15 gm. of long colorless needles, m.p. 152–153°.

$C_{16}N_{20}O_4N_2S$.	Calculated.	C 57.1, H 6.0, N 8.3
	Found.	" 57.5, " 6.2, " 8.0

Taurylaminocyclohexane—The phthalimido compound was hydrolyzed with hydrazine hydrate and hydrochloric acid, as described for the preparation of tauryl-2-aminopyridine. The taurylaminocyclohexane was obtained in quantitative yield by neutralization of the hygroscopic hydrochloride followed by evaporation of the aqueous solution and extraction of the residue with ethanol. On recrystallization of the crude product from ethanol, there was obtained a colorless product, m.p. 92–93°.

d Pantoyltaurylaminocyclohexane (*VI*), (*SN 3281*)—To 6.2 gm. of taurylaminocyclohexane were added 4 gm. of *l*-pantolactone, and the mixture heated at 120° for 4 hours. The resulting oil was solidified by dissolving it in acetone and pouring the solution into dry ether, but it could not be induced to crystallize. There were thus obtained 9 gm. of the crude product, which was used for testing. A portion of this product was dissolved in a small amount of water, and this solution, after standing for several days, slowly deposited colorless crystals, which were filtered off and recrystallized several times from water plus a few drops of ethanol, to give colorless needles, m.p. 125–126°. $[\alpha]_D^{23} = +2.7°$ (67 mg. in 2.0 ml. of water).

$C_{14}H_{28}O_5N_2S$.	Calculated.	C 50.0, H 8.4, N 8.3
	Found.	" 50.5, " 8.3, " 8.3

β-Phthalimidoethanesulfonylaminooctadecane—To a solution of 20 gm. of β-phthalimidoethanesulfonyl chloride dissolved in the minimum amount of benzene was added a solution of 41 gm. of octadecylamine in benzene. The solution was refluxed for 1 hour and then evaporated to dryness under reduced pressure. The residue (about 50 gm.) was recrystallized from ethanol to give 33 gm. of colorless plates, m.p. 109–109.5°.

$C_{28}H_{46}O_4N_2S$. Calculated. C 66.4, H 9.2, N 5.5
Found. " 66.4, " 9.3, " 5.7

The alcoholic mother liquors were evaporated almost to dryness and poured into a large amount of ether, whereupon the octadecylamine hydrochloride precipitated in a fairly pure form, m.p. 155–160°.

Taurylaminooctadecane—The hydrolysis of the phthalimido compound was carried out with hydrazine hydrate, as described for the preparation of tauryl-2-aminopyridine. The combined precipitate of phthalhydrazide and taurylaminooctadecane hydrochloride was centrifuged down, and, after removal of the excess hydrochloric acid by several washings with water followed by recovery of the precipitate by centrifuging, the solids were treated with two successive portions of normal sodium hydroxide solution. The product from this treatment was filtered, washed thoroughly with water, and dried over sulfuric acid. The resulting solid was twice crystallized from ethanol to give 16 gm. of colorless needles, m.p. 90–91°. The basic filtrate and washings could be acidified to recover the phthalhydrazide.

$C_{20}H_{44}O_2N_2S$. Calculated, N 7.2; found, N 7.2

d-Pantoyltaurylaminooctadecane (*VII*), (*SN 3282*)—To 19.8 gm. of the above taurylaminooctadecane were added 7.5 gm. of *l*-pantolactone, and the mixture heated at 100° for 2 hours. The resulting solid was recrystallized from ethanol to give 23 gm. of colorless prisms, m.p. 98–100°. $[\alpha]_D^{23} = +51.0°$ (46 mg. in 5.0 ml. of chloroform).

$C_{26}H_{54}O_5N_2S$. Calculated. C 61.7, H 10.7, N 5.5
Found. " 61.7, " 10.8, " 5.6

Attempted Preparation of Pantoyltauryl-9-aminoanthracene (*SN 5923*)—Although the instability of certain of the intermediates and of the final product has precluded satisfactory analyses, we wish to record the results obtained. 9-Nitroanthracene (10) was prepared and reduced to 9-aminoanthracene (11, 12) with stannous chloride. The compound had a melting point of 148–151° but decomposed slowly on standing in a closed vessel. The anthramine was condensed with β-phthalimidoethanesulfonyl chloride in pyridine to give β-phthalimidoethanesulfonyl-9-aminoanthracene (?) as a light buff-colored solid which did not melt below 300°. The phthalimido compound was hydrolyzed with hydrazine hydrate and the resulting tauryl-9-aminoanthracene hydrochloride (?) had a melting point of 205–206° (with decomposition) when recrystallized from ethanol. The hydrochloride was neutralized with sodium bicarbonate and the free base recrystallized from ethanol to give light buff-colored crystals, m.p. 174–175°. The free base gradually decomposed on standing and was therefore immediately condensed with *l*-pantolactone by heating for 3 hours at 120°. The

product was crystallized from cellosolve and light yellow crystals of pantoyltauryl-9-aminoanthracene (?), m.p. 219–220°, were obtained. Recrystallization from cellosolve or prolonged standing at room temperature was accompanied by decomposition, as evidenced by darkening. The analyses gave results too variable to be of significance.

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SUMMARY

The preparation is described of a series of optically active derivatives of pantoyltaurine, in as pure a form as possible, for testing for antimalarial activity.

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