THE INHIBITORY ACTION OF THE MONOETHYL ESTERS OF *S-n*-ALKYLTHIOMALIC ACIDS AND THE MONOETHYL ESTERS OF ALKANE SULPHONYL SUCCINIC ACIDS ON THE GROWTH OF *MYCOBACTERIUM TUBERCULOSIS*

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Recently Barry & Twomey (1947) have described the synthesis of various half-esters of monoalkyl succinic acids. Their inhibitory action on the growth of *Mycobacterium tuberculosis* and *M. smegmatis* and other organisms has been described by the author (1947). Because the work of Feldman and his associates with sulphone compounds showed results of much interest (1946), Barry, O'Rourke & Twomey (1947) prepared compounds incorporating a sulphone grouping in a molecular structure of established effectiveness *in vitro* against the *M. tuberculosis* and other organisms. A series of alkylthiomalic acids was synthesized:

These, on oxidation, could be readily converted to the corresponding alkane sulphonyl succinic acids:

Six alkylthiomalic acids and six alkane sulphonyl succinic acids in all were prepared, and they showed only a very slight inhibitory effect on the growth *in vitro* of the *M. tuberculosis*.

Monoethyl esters of the alkylthiomalic acids were then prepared by refluxing the anhydrides for a few hours with dry ethyl alcohol and the solutions were neutralized exactly with standard sodium hydroxide; after removal of the alcohol, under reduced pressure, they were made up to a known concentration. Solutions of the sodium salts of the half-esters prepared in this way were used for the *in vitro* tests. The half-esters of the alkane sulphonyl succinic acids were prepared by oxidation of the half-esters of the alkylthiomalic acids:

These compounds were tested for their inhibitory action on the growth of *M. tuberculosis* and *M. smegmatis*, and also for their haemolytic action in standard suspensions of washed human red blood cells (Tables 1 and 2).

METHODS

The medium used was nutrient peptone digest broth to which the chemicals were added in the desired concentrations. The organisms used were *M. smegmatis* (National Collection of Type Cultures) and *M. tuberculosis* H37 Rv (Trudeau). After repeated subculturing these grew well in the above medium. Uniform inocula were used by making the bacterial suspensions as even as possible and bringing to a standard density; each inoculum of *M. tuberculosis* weighed ca. 0.005 mg. Final readings of the growth of *M. smegmatis* were taken on the 6th day and of the *M. tuberculosis* on the 42nd day of incubation and compared with the controls. The chemicals were also tested for haemolytic action on standard suspensions of washed human red blood cells at 37° C. for 1 hr.

RESULTS

Tables 1 and 2 summarize the results of the *in vitro* experiments with these compounds. It is seen in Table 1 that the optimum antibacterial effect is reached when the alkyl residue contains 12-14 carbon atoms, and Table 2 shows that a longer chain (hexadecane) is required to produce optimum antibacterial effect in this series.

Effect of serum on antibacterial and haemolytic activity

The addition of pooled human serum, 5 % to the medium, reduced the effectiveness of these compounds against bacterial growth on an average about five times; and the addition of 10 % normal human serum raised the concentration of these compounds, necessary for haemolysis, on the average about a hundred times. This action of human serum is probably due to the formation of a stoichiometric complex between the chemicals and the serum albumin.

Table 1. *Antibacterial and haemolytic actions of the half-esters of S-n-alkylthiomalic acids*

Half-esters	Biological activity		
	Sm.	M.tbc.	Haem. index
Nonyl thiomalic acid	2,500	35,000	20,000
Undecyl thiomalic acid	9,000	45,000	50,000
Dodecyl thiomalic acid	15,000	150,000	100,000
Tetradecyl thiomalic acid	35,000	130,000	100,000
Hexadecyl thiomalic acid	18,000	120,000	200,000
Octadecyl thiomalic acid	2,500	30,000	200,000

Sm. = dilution of the sodium salt of the half-ester which causes complete inhibition of growth of *M. smegmatis* for 6 days.

M.tbc. = dilution of the sodium salt of the half-ester which causes complete inhibition of growth of *M. tuberculosis* H37 Rv for 42 days.

Haem. index = dilution of the half-ester which produces haemolysis in a suspension of washed human red blood cells.

Table 2. *Antibacterial and haemolytic actions of the half-esters of alkane sulphonyl succinic acids*

Half-esters	Biological activity		
	Sm.	M.tbc.	Haem. index
Nonane sulphonyl succinic acid	1,000	5,000	20,000
Undecane sulphonyl succinic acid	4,000	20,000	50,000
Dodecane sulphonyl succinic acid	8,000	18,000	100,000
Tetradecane sulphonyl succinic acid	10,000	35,000	200,000
Hexadecane sulphonyl succinic acid	20,000	75,000	200,000
Octadecane sulphonyl succinic acid	12,000	35,000	200,000

Sm. = dilution of the sodium salt of the half-ester which causes complete inhibition of the growth of *M. smegmatis* for 6 days.

M.tbc. = dilution of the sodium salt of the half-ester which causes complete inhibition of growth of *M. tuberculosis* H37 Rv for 42 days.

Haem. index = dilution of the half-ester which produces haemolysis in a suspension of washed human red blood cells.

SUMMARY

1. A new series of compounds has been found which markedly inhibits the growth of *Mycobacterium tuberculosis* and *M. smegmatis*.

2. These substances are strongly haemolytic against human red blood cells.

3. The effectiveness of the antibacterial and haemolytic actions depends upon the length of the alkyl chain.

4. The antibacterial and haemolytic actions are reduced by human serum.

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