[Contribution from the Chemical Laboratory of the University of Illinois]

THE PREPARATION OF CERTAIN OCTADECANOIC ACIDS AND THEIR BACTERICIDAL ACTION TOWARD B. LEPRAE. XV[1]

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In previous papers numerous series of organic acids of the general formula $RCH(CO_2H)R'$ in which R is a cyclohexyl, cyclopentyl, cyclopentenyl, or cyclopropyl group, or in which R is one of these groups substituted in the ω-position of the alkyl group, and in which R′ is an alkyl group, have been prepared and studied for their bactericidal action toward *B. leprae.* The results indicated that there are no very marked differences between the acids containing the 3, 5 or 6-membered rings, and consequently the supposition that a ring is an important factor for the bactericidal action is probably incorrect. The acids which gave the best results in each of the series studied were those containing from sixteen to eighteen carbon atoms. When the two substituted groups in the acetic acids were of approximately the same number of carbon atoms, the products in general had the most pronounced bactericidal action.

The object of the present investigation was to determine whether acids not containing a ring might have a bactericidal action. A number of isomeric octadecanoic acids were synthesized which included a complete series of compounds with a chain of seventeen carbon atoms and a carboxyl group substituted in every possible position. The bactericidal properties are reported in Table I.

The results indicate conclusively that no ring in the molecule is necessary for bactericidal action. The marked difference between the acid with the carboxyl at the end of the chain and those with the carboxyl at any other position is particularly to be noticed. The individual members of the series do not show as regular a variation in bactericidal properties with change in constitution as was found in the previous series containing the rings. In fact, the variability resembles to a certain extent that found in the previously prepared ring-containing acids which had nineteen

[1] For previous articles in this field see (a) Shriner and Adams, This Journal, **47**, 2727 (1925); (b) Noller with Adams, *ibid.*, **48**, 1080 (1926); (c) Hiers with Adams, *ibid.*, **48**, 1089 (1926); (d) **48**, 2385 (1926); (e) Van Dyke and Adams, *ibid.*, **48**, 2393 (1926); (f) Sacks with Adams, *ibid.*, **48**, 2395 (1926); (g) Noller and Adams, *ibid.*, **48**, 2444 (1926); (h) Adams, Stanley, Ford and Peterson, *ibid.*, **49**, 2934 (1927); (i) Arvin with Adams, *ibid.*, **49**, 2940 (1927); (j) Adams, Stanley and Stearns, *ibid.*, **50**, 1475 (1928); (k) Yohe and Adams, *ibid.*, **50**, 1503 (1928); (l) Arvin and Adams, *ibid.*, **50**, 1983 (1928); (m) Davies and Adams, *ibid.*, **50**, 2297 (1928).

[2] This communication is an abstract of portions of theses submitted by W. M. Stanley in partial fulfilment of the requirements for the degree of Doctor of Philosophy and by Marian S. Jay in partial fulfilment of the requirements for the degree of Master of Science in Chemistry at the University of Illinois.

TABLE I

BACTERICIDAL ACTION OF OCTADECANOIC ACIDS

	Dilutions of sodium salts in thousands											
	5	15	25	50	62	74	85	100	125	155	192	250
$C_{17}H_{35}CO_2H$	+	+	+	+	+	+	+	+	+	+	+	+
$CH_3CH(CO_2H)C_{15}H_{31}$	−	−	−	−	−	±	±	±	±	+	+	+
$C_2H_5CH(CO_2H)C_{14}H_{29}$	−	−	−	−	−	−	−	−	−	−	±	+
$C_3H_7CH(CO_2H)C_{13}H_{27}$	±	±	+	+	+	+	+	+	+	+	+	+
$C_4H_9CH(CO_2H)C_{12}H_{25}$	−	±	±	+	+	+	±	+	+	+	+	+
$C_5H_{11}CH(CO_2H)C_{11}H_{23}$	−	−	±	±	+	+	+	+	+	+	+	+
$C_6H_{13}CH(CO_2H)C_{10}H_{21}$	−	−	−	−	−	±	±	+	+	+	+	+
$C_7H_{15}CH(CO_2H)C_9H_{19}$	−	−	−	−	−	±	±	±	±	+	+	+
$C_8H_{17}CH(CO_2H)C_8H_{17}$	−	−	−	−	−	±	−	±	+	+	+	+
$(CH_3)_2CHCH(CO_2H)C_{13}H_{27}$	±	+	+	+	+	+	+	+	+	+	+	+
$(CH_3)_2CHCH_2CH(CO_2H)C_{12}H_{25}$	−	−	−	±	±	+	+	+	+	+	+	+
$C_2H_5CH(CH_3)CH(CO_2H)C_{12}H_{25}$	−	−	−	−	−	−	±	+	+	+	+	+
$C_8H_7CH(CH_3)CH(CO_2H)C_{11}H_{23}$	−	−	−	−	−	±	+	+	+	+	+	+

to twenty-one carbon atoms. It seemed probable, therefore, that analogous acids of somewhat lower molecular weight, such as hexadecanoic acids, should prove more effective. A complete series of acids was synthesized which contained a chain of fifteen carbon atoms with the carboxyl in each possible position. The bacteriological results are given in Table II.

TABLE II

BACTERICIDAL ACTION OF HEXADECANOIC ACIDS

	Dilutions of sodium salts in thousands											
	5	15	25	50	62	74	85	100	125	155	192	250
$C_{15}H_{31}CO_2H$	+	+	+	+	+	+	+	+	+	+	+	+
$CH_3CH(CO_2H)C_{13}H_{27}$	−	−	−	−	±	±	+	+	+	+	+	+
$C_2H_5CH(CO_2H)C_{12}H_{25}$	−	−	−	−	−	−	−	−	±	+	+	+
$C_3H_7CH(CO_2H)C_{11}H_{23}$	−	−	−	−	−	−	±	±	+	+	+	+
$C_4H_9CH(CO_2H)C_{10}H_{21}$	−	−	−	−	−	−	−	−	−	±	±	+
$C_5H_{11}CH(CO_2H)C_9H_{19}$	−	−	−	−	−	−	−	±	±	+	+	+
$C_6H_{13}CH(CO_2H)C_8H_{17}$	−	−	−	−	−	−	−	−	−	−	−	+
$C_7H_{15}CH(CO_2H)C_7H_{15}$	−	−	−	−	−	−	−	−	−	−	±	+
$(CH_3)_2CHCH_2CH(CO_2H)C_{10}H_{21}$	−	−	−	−	−	−	−	−	−	−	±	±
$C_2H_5CH(CH_3)CH(CO_2H)C_{10}H_{21}$	−	−	−	−	−	−	−	±	+	+	+	+

Analysis of the data indicates that the prediction was correct. The acids were in practically every instance more bactericidal than the octadecanoic acid homologs. Moreover, the regularity in the change of bactericidal property with constitution, which did not exist in the acids of eighteen carbon atoms, is apparent here. The marked difference between the acid with the carboxyl at the end of the chain and acids with the carboxyl in any other position again deserves notice.

The conclusion from the studies of the bactericidal effect toward acid-fast bacteria, and especially toward *B. leprae*, of the large number of acids described in this series of papers, is that the effect can hardly be attributed to a chemical specificity of the individual acids but must, probably, be due to a combination of physical properties common to many of these acids. The mechanism of the bactericidal effect may be looked upon as an adsorption or solution of the acid in the waxy coating of the bacteria, with a consequent smothering or suffocation of the organism. The physical properties of the effective acids are being studied in an attempt to correlate physical properties and bactericidal action.

Although occasional tests have been made to determine the bactericidal effect of some of these acids toward *B. smegmatis* and *B. tuberculosis*, no report of the results has as yet been published. The relative bactericidal action for *B. tuberculosis* and *B. leprae* of a few selected acids is recorded in Table III.

TABLE III

BACTERIOLOGICAL ACTION OF CERTAIN ACIDS TO *B. tuberculosis*

	Dilution of sodium salts in thousands 1	5	10	16	20	25	33	50	100	Max. eff. diln. *vs.* *B. leprae* in thousands
$C_7H_{15}CH(CO_2H)C_7H_{15}$	−	−	−	−	−	−	−	±	+	155
$C_6H_{11}(CH_2)_2CH(CO_2H)CH_2C_6H_{11}$	−	−	−	−	−	−	−	−	+	100
$C_6H_{11}(CH_2)_2CH(CO_2H)C_7H_{15}$	−	−	−	−	−	−	−	±	+	220
$C_6H_{11}CH(CO_2H)C_{10}H_{21}$	−	−	−	−	−	−	−	±	+	180
$C_5H_7(CH_2)_2CH(CO_2H)C_{10}H_{21}$	−	−	−	−	±	+	+	+	+	192
$C_6H_{11}(CH_2)_3CH(CO_2H)(CH_2)_3C_6H_{11}$	−	+								25
$C_6H_{11}(CH_2)_4CH(CO_2H)(CH_2)_2C_6H_{11}$	−	+								25

It is obvious that the acids are in general only a fraction as effective toward *B. tuberculosis* as they are toward *B. leprae;* nevertheless they do have a similar action. The acids of too high molecular weight have a relatively low effect just as was found in the tests against *B. leprae.*

TABLE IV

BACTERICIDAL TESTS TOWARD VARIOUS STRAINS OF *B. leprae*

	No. of strain	Dilution of sodium salts in thousands 10	25	50	75	100	120	142	160	182	200	221	250
	1	−	−	−	−	−	±	±	+	+	+	±	+
	2	−	−	−	±	−	±	+	+	+	+	+	+
	3	−	−	+	+	+	+	+	+	+	+	+	+
Mixed acids of	4	−	−	−	−	±	±	−	−	−	−	+	+
Hydnocarpus Alcalae	5	−	−	±	−	−	+	±	+	±	+	+	+
	6	−	−	+	+	+	+	+	+	+	+	+	+
	7	−	±	+	+	+	+	±	+	+	+	+	+
	8	−	−	−	±	+	+	+	+	+	+	+	+
	1	−	−	−	−	−	−	±	+	+	+	+	+
	2	−	−	−	−	−	+	±	±	±	+	+	+
	3	−	−	−	−	−	±	+	+	+	+	+	+
Mixed acids of	4	−	−	−	−	±	−	−	−	−	+	±	+
Chaulmoogra Oil	5	−	−	−	−	−	−	−	±	+	+	+	+
	6	−	−	−	±	±	+	±	+	+	+	+	+
	7	−	−	−	±	±	+	+	+	+	+	+	+
	8	−	−	−	−	−	±	+	+	+	+	+	+

Table IV (*Concluded*)

	No. of strain	10	25	50	75	100	120	142	160	182	200	221	250
		Dilution of sodium salts in thousands											
	1	−	−	−	−	±	+	+	+	+	+	+	+
	2	−	−	−	−	+	+	+	+	+	+	+	+
	3	−	−	−	−	+	+	+	+	+	±	+	+
$C_5H_7CH(CO_2H)C_9H_{19}$	4	−	−	−	−	−	−	−	−	−	−	−	±
	5	−	−	−	−	−	−	−	±	+	+	±	+
	6	−	−	±	+	+	+	+	+	+	+	+	+
	7	−	−	−	−	+	+	+	+	+	+	+	+
	8	−	−	−	−	+	+	+	+	+	+	+	+
	1.	−	−	−	−	−	−	−	−	−	−	−	±
	2	−	−	−	−	−	−	−	−	−	±	±	±
	3	−	−	−	−	−	−	−	−	−	−	−	+
$C_5H_9(CH_2)_2CH(CO_2H)C_8H_{17}$	4	−	−	−	−	−	−	−	−	−	−	±	±
	5	−	−	−	−	−	−	−	−	−	±	+	+
	6	−	−	−	−	−	−	−	−	−	−	±	+
	7	−	−	−	−	−	−	−	−	−	±	±	+
	8	−	−	−	−	−	−	−	−	−	±	+	+
	1	−	−	−	−	−	−	−	−	−	−	−	±
	2	−	−	−	±	±	+	+	+	+	+	+	+
	3	−	−	−	−	−	−	−	−	−	−	±	±
$C_6H_{11}(CH_2)_2CH(CO_2H)C_8H_{17}$	4	−	−	−	−	−	−	−	−	−	−	−	−
	5	−	−	−	−	−	−	−	−	−	−	−	+
	6	−	−	−	−	−	−	−	−	−	−	±	+
	7	−	−	−	−	−	−	−	−	−	−	±	±
	8	−	−	−	−	−	−	−	−	±	+	+	+

Strain 1 is a culture of *B. leprae* of unknown history obtained from the University of Chicago. It has been cultured on laboratory media for several years and is a very rapidly-growing strain. All of the tests made on the various acids have been with this particular organism. Strains 2 to 8 inclusive were obtained from the American Type Culture Collection and are listed as follows: No. 2, 65 Hyg. Lab., Washington, D. C., 365—"Duval," 1925; No. 3, 66 Hyg. Lab., Washington, D. C., 367—"rat 2," 1925; No. 4, 67 Hyg. Lab., Washington, D. C., 368—"rat 12, Dr. McCoy," 1925; No. 5, 68 Hyg. Lab., Washington, D. C., 361—"Needham," 1925; No. 6, 69 Army Med. School, Washington, D. C., 366—"rat 1," 1925; No. 7, 282 of American Type Culture Collection; No. 8, 283 of American Type Culture Collection.

In order to be certain that the acids are bactericidal toward strains of *B. leprae* other than that regularly used, a few tests were made and are recorded in Table IV. Three synthetic acids and the mixed acids from two natural oils were studied, using eight different strains of bacteria.

It can be seen that the synthetic acids are much more bactericidal than the mixed acids of the natural oils, and that the results run parallel with the different, more or less resistant, strains. Strain 4 is obviously much more easily killed than the others and too much significance should, therefore, not be attached to the results on this particular one.

The synthetic acids were prepared from the proper disubstituted malonic esters by saponification and decomposition of the malonic acids.

Experimental

The general methods of preparation of the various intermediates have been described in previous articles in this series.

TABLE V

DIETHYL DIALKYL MALONATES (INTERMEDIATES FOR C_{18} ACIDS)

	B. p., °C.	n_D^{25}	d_4^{25}	Found,[a] % C	H
$CH_3C(CO_2C_2H_5)_2C_{15}H_{31}$	179–183 (5 mm.)	1.4453	0.9119	71.71	11.38
$C_2H_5C(CO_2C_2H_5)_2C_{14}H_{29}$	172–177 (3 mm.)	1.4461	.9163	71.67	11.52
$C_3H_7C(CO_2C_2H_5)_2C_{13}H_{27}$	183–187 (5 mm.)	1.4475	.9048	71.66	11.36
$C_4H_9C(CO_2C_2H_5)_2C_{12}H_{25}$	175–180 (3.5 mm.)	1.4473	.9104	71.48	11.33
$C_5H_{11}C(CO_2C_2H_5)_2C_{11}H_{23}$	180–185 (5 mm.)	1.4509	.9124	72.01	11.32
$C_6H_{13}C(CO_2H_5)_2C_{10}H_{21}$	185–188 (2.5 mm.)	1.4476	.9118	71.27	11.40
$C_7H_{15}C(CO_2C_2H_5)_2C_9H_{19}$	193–197 (5 mm.)	1.4471	.9118	71.59	11.47
$C_8H_{17}C(CO_2C_2H_5)_2C_8H_{17}$	192–195 (3 mm.)	1.4471	.9135	71.35	11.58
$(CH_3)_2CHC(CO_2C_2H_5)_2C_{13}H_{27}$	179–183 (5 mm.)	1.4491	.9144	71.59	11.38
$(CH_3)_2CHCH_2C(CO_2C_2H_5)_2C_{12}H_{25}$	180–185 (5 mm.)	1.4481	.9115	71.51	11.74
$C_2H_5CH(CH_3)C(CO_2C_2H_5)_2C_{12}H_{25}$	180–184 (5 mm.)	1.4501	.9163	72.01	11.31
$C_3H_7CH(CH_3)C(CO_2C_2H_5)_2C_{11}H_{23}$	175–178 (4 mm.)	1.4509	.9155	71.73	11.34

[a] Calcd. for $C_{23}H_{44}O_4$: C, 71.81; H, 11.54.

TABLE VI

DIETHYL DIALKYL MALONATES (INTERMEDIATES FOR C_{16} ACIDS)

	B. p., °C.	n_D^{25}	d_4^{25}	Found,[a] % C	H
$CH_3C(CO_2C_2H_5)_2C_{13}H_{27}$	167–170 (3 mm.)	1.4418	0.9181	70.48	11.16
$C_2H_5C(CO_2C_2H_5)_2C_{12}H_{25}$	181–183 (4 mm.)	1.4422	.9249	70.40	11.29
$C_3H_7C(CO_2C_2H_5)_2C_{11}H_{23}$	178–179 (4 mm.)	1.4422	.9186	70.50	11.22
$C_4H_9C(CO_2C_2H_5)_2C_{10}H_{21}$	181–183 (4 mm.)	1.4424	.9220	70.81	11.20
$C_5H_{11}C(CO_2C_2H_5)_2C_9H_{19}$	185–186 (5 mm.)	1.4462	.9282	70.45	11.00
$C_6H_{13}C(CO_2C_2H_5)_2C_8H_{17}$	175–178 (4 mm.)	1.4458	.9168	70.38	11.18
$C_7H_{15}C(CO_2C_2H_5)_2C_7H_{15}$	178–180 (3 mm.)	1.4459	.9169	70.50	11.20
$(CH_3)_2CHCH_2C(CO_2C_2H_5)_2C_{10}H_{21}$	160–162 (2 mm.)	1.4428	.9207	70.51	11.00
$C_2H_5CH(CH_3)C(CO_2C_2H_5)_2C_{10}H_{21}$	196–198 (10 mm.)	1.4454	.9253	70.70	11.01

[a] Calcd. for $C_{21}H_{40}O_4$: C, 70.78; H, 11.24.

TABLE VII

OCTADECANOIC ACIDS

	M. p., °C.	B. p., °C.	n_D^{25}	d_4^{25}	Found,[a] % C	H
$CH_3CH(CO_2H)C_{15}H_{31}$[b]	34–35	179–183 (5 mm.)	....	....	76.20	12.54
$C_2H_5CH(CO_2H)C_{14}H_{29}$	23–24	167–170 (2.5 mm.)	1.4531	0.8767	76.03	12.55
$C_3H_7CH(CO_2H)C_{13}H_{27}$	31–32	179–183 (5 mm.)	....	....	75.90	12.46
$C_4H_9CH(CO_2H)C_{12}H_{25}$	23–24	180–184 (4 mm.)	1.4528	.8743	76.03	12.54
$C_5H_{11}CH(CO_2H)C_{11}H_{23}$	...	180–185 (4 mm.)	1.4519	.8829	76.21	12.48
$C_6H_{13}CH(CO_2H)C_{10}H_{21}$[c]	...	182–184 (5 mm.)	1.4527	.8741	75.79	12.73
$C_7H_{15}CH(CO_2H)C_9H_{19}$[c]	...	180–183 (5 mm.)	1.4528	.8747	75.92	12.83
$C_8H_{17}CH(CO_2H)C_8H_{17}$[d]	35–36	183–185 (5 mm.)	....	....	76.00	12.75
$(CH_3)_2CHCH(CO_2H)$-$C_{13}H_{27}$	58–59	178–182 (5 mm.)	....	....	76.11	12.51

Table VII (*Concluded*)

	M. p., °C.	B. p., °C.	n_D^{25}	d_4^{25}	Found,[a] % C	H
$(CH_3)_2CHCH_2CH(CO_2H)$-$C_{12}H_{25}$	26–27	175–180 (4 mm.)	....	....	76.02	12.52
$C_2H_5CH(CH_3)CH(CO_2H)$-$C_{12}H_{25}$	38–39	178–183 (6 mm.)	....	....	75.82	12.56
$C_3H_7CH(CH_3)CH(CO_2H)$-$C_{11}H_{23}$	37–38	175–178 (5 mm.)	....	....	76.24	12.58

[a] Calcd. for $C_{18}H_{36}O_2$: C, 75.98; H, 12.76.

[b] This acid has been previously described and is reported as having m. p. 51–51.5° [Morgan and Holmes, *J. Soc. Chem. Ind.*, **46**, 152 (1927)].

[c] These acids and the necessary intermediates were prepared by Gerald H. Coleman.

[d] This acid has been previously described and is reported as having m. p. 38.5°, b. p. 270–275 (100 mm.) [M. Guthzeit, *Ann.*, **204**, 11 (1880)].

Table VIII

Hexadecanoic Acids

	B. p., °C.	M. p., °C.	n_D^{25}	d_4^{25}	Found,[a] % C	H
$CH_3CH(CO_2H)C_{13}H_{27}$[b]	172–173 (2.5 mm.)	24	1.4453	0.8765	74.74	12.48
$C_2H_5CH(CO_2H)C_{12}H_{25}$	178–179 (3 mm.)	23	1.4460	.8808	75.20	12.50
$C_3H_7CH(CO_2H)C_{11}H_{23}$	178–179 (3 mm.)	16.5–17	1.4460	.8808	74.65	12.49
$C_4H_9CH(CO_2H)C_{10}H_{21}$	175–176 (3 mm.)	13–14	1.4458	.8789	74.60	12.42
$C_5H_{11}CH(CO_2H)C_9H_{19}$	178–179 (3 mm.)	9–10	1.4518	.8887	74.90	12.30
$C_6H_{13}CH(CO_2H)C_8H_{17}$[c]	165–168 (2 mm.)	.....	1.4495	.8768	74.82	12.56
$C_7H_{15}CH(CO_2H)C_7H_{15}$[c,d]	187–189 (4 mm.)	26–27	1.4497	.8771	74.72	12.56
$(CH_3)_2CHCH_2CH$-$(CO_2H)C_{10}H_{21}$	187–188 (9 mm.)	17.5–18	1.4448	.8763	75.30	12.70
$C_2H_5CH(CH_3)CH$-$(CO_2H)C_{10}H_{21}$	185–186 (9 mm.)	38–39	....	....	74.72	12.56

[a] Calcd. for $C_{16}H_{32}O_2$: C, 75.00; H, 12.70.

[b] This acid has been previously described and is reported as having m. p. 48° [Morgan and Holmes, *J. Soc. Chem. Ind.*, **46**, 152 (1927)].

[c] These acids and the necessary intermediates were prepared by Gerald H. Coleman.

[d] This acid has been previously described and is reported as having m. p. 26–27°, b. p. 240–250° (85 mm.) [F. Jourdan, *Ann.* **200**, 116 (1880)].

Summary

1. It has been demonstrated that a ring structure is unnecessary in order that certain various organic acids have a bactericidal action.

2. A number of octadecanoic acids and hexadecanoic acids have been synthesized and their bactericidal actions determined.

3. A table of bactericidal values toward *B. tuberculosis* of a few selected acids is included. The effect is much less than toward *B. leprae*.

4. A table of bactericidal values of a few acids toward eight different strains of *B. leprae* is given.

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