of lesser magnitude was observed at a P_H of 7.73, using a low concentration of the secondary phosphate-sodium hydroxide buffer. But both solutions were poorly buffered, because the addition of the 1% protein changed the original P_H of the buffer by more than one P_H unit; therefore, since at P_H 7.56 a solution well buffered with primary-secondary buffer showed no hydration, this hydration effect is perhaps repressed if the solution is properly buffered. When more data have been collected this point can be decided.

The condition of the hemoglobin at the extremes of P_H and a fuller discussion of the phenomena occurring will be taken up in a later paper.

The rather high expenses connected with the construction of this centrifuge have been defrayed by grants from the foundation "Therese och Johan Anderssons Minne" and from the Nobel Fund for Chemistry.

Summary

1. An oil turbine type of ultracentrifuge has been described capable of running at a speed of 42,000 r.p.m. and yielding a centrifugal force 104,000 times that of gravity.

2. Determinations of the influence of P_H on the diffusion constant, molecular weight and specific sedimentation velocity of carbon monoxide-hemoglobin are reported over a P_H range 5.4–10.2. The diffusion constant and the specific sedimentation velocity are normal, respectively, 0.071 cm.^{2}/day and 5.46×10^{-13} cm./sec. at 30° over the range of P_H 6.0–7.56, and the molecular weight is normal, 68,000, at least from a P_H of 6.0 to 9.05.

3. At a P_H of 9.05, in the neighborhood of a maximum in the partial specific volume curve, the Hb molecule appears to hold a monomolecular layer of water at its surface.

UPSALA, SWEDEN

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF ILLINOIS]

VARIOUS ω-CYCLOHEXYLALKYL ALKYL ACETIC ACIDS AND THEIR ACTION TOWARD B. LEPRAE. VIII[1]

BY ROGER ADAMS, W. M. STANLEY, S. G. FORD AND W. R. PETERSON[2]

RECEIVED AUGUST 29, 1927 PUBLISHED NOVEMBER 5, 1927

In an earlier paper the ω-cyclohexyl derivatives of various normal aliphatic acids containing from one to thirteen carbon atoms in the side chain were described and their bactericidal character toward *B. Leprae*

[1] For previous articles in this field see (a) Shriner and Adams, THIS JOURNAL, **47**, 2727 (1925); (b) Noller with Adams, *ibid.*, **48**, 1074 (1926); (c) **48**, 1080 (1926); (d) Hiers with Adams, *ibid.*, **48**, 1089 (1926); (e) Van Dyke and Adams, *ibid.*, **48**, 2393 (1926); (f) Sacks with Adams, *ibid.*, **48**, 2395 (1926); (g) Hiers with Adams, *ibid.*, **48**, 2385 (1926).

[2] This communication is an abstract of the theses submitted by W. M. Stanley, S. G. Ford and W. R. Peterson in partial fulfilment of the requirements for the degree of Master of Science in Chemistry at the University of Illinois.

and other acid-fast was bacteria tested. The acid with the three-carbon side chain showed a very slight action, but with increase in the length of the side chain the bactericidal character increased until a maximum was reached in the molecule with a nine-carbon side chain (I)

$$C_6H_{11}(CH_2)_xCO_2H$$
I

This investigation had as its object the synthesis of acids isomeric with those previously studied, containing the cyclohexane ring with side chains of varying length but with the carboxyl in positions other than at the end of the chain. From bactericidal tests it could then be determined whether the position of the carboxyl group at the end of the chain as in the natural acids is important, or whether the chief function of the carboxyl may possibly be the solubilizing of the molecule. Moreover, the new acids, if bactericidal, would lead to further conclusions in regard to the significance of molecular weight either of the whole molecule or of the side chain.

In this communication three series of acids have been described: β-cyclohexylethyl alkyl (II), γ-cyclohexylpropyl alkyl (III), δ-cyclohexylbutyl (IV) alkyl acetic acids, and the alkyl group has been varied in series (II) from ethyl to *n*-octyl, in series (III) from ethyl to *n*-heptyl and in series (IV) from ethyl to *n*-hexyl. Three isomers of each acid containing in the side chain from eight to twelve carbon atoms and holding the carboxyl group on the third, fourth and fifth carbon atoms from the ring, were thus made available in addition to a number of acids of lower molecular weight.

$C_6H_{11}(CH_2)_2CH(CO_2H)R$ II $C_6H_{11}(CH_2)_3CH(CO_2H)R$ III $C_6H_{11}(CH_2)_4CH(CO_2H)R$ IV

These acids were tested against the same strain of *B. Leprae* as that used in the earlier work (Table I). In series (II) the compounds with the R group as ethyl or propyl showed only a slight action, but with the *n*-butyl group they killed in a dilution of 1:40,000. The action rapidly increased with the size of the molecule until the *n*-heptyl and *n*-octyl showed a bactericidal action in dilutions of 1:220,000 and 1:320,000, respectively, far greater than the sodium salts of chaulmoogric or hydnocarpic acids or of the mixed acids from the saponified natural oils now used in the treatment of leprosy or of the ω-cyclohexyl aliphatic acids with the carboxyl at the end of the chain. The same results were obtained in series (III) and series (IV), the bactericidal action increasing very rapidly with the size of the molecule and the compounds isomeric with those in series (II) giving approximately the same bactericidal effect in the same dilutions. It is obvious that the position of the carboxyl group is probably of secondary importance. The value of this fact cannot be underestimated, because all of the most effective acids described in this paper are easily prepared as compared with the effective ones in which the carboxyl group is at the

end of the side chain. Moreover, investigation of the effect of other groups in the molecule such as various types of rings, amino groups, halogens, double bonds, etc., is rendered much easier in acids of this type.

TABLE I

BACTERIOLOGICAL TESTS TO *B. Leprae*

	Dilutions of sodium salts																										
	10,000	20,000	30,000	40,000	50,000	60,000	70,000	80,000	90,000	100,000	110,000	120,000	130,000	140,000	150,000	160,000	170,000	180,000	190,000	200,000	220,000	240,000	260,000	280,000	300,000	320,000	340,000
Cyclohexylethyl alkyl acetic acids, $C_6H_{11}(CH_2)_2CH(COOH)R$. R =																											
C_2H_5	−	+	±	±	−	±	+	±	+	+																	
n-C_3H_7	−	±	+	+	+	±	±	±	+	+																	
n-C_4H_9	−	−	−	−	+	+	+	+	+	+																	
n-C_5H_{11}	−	−	−	±	±	±	+	±	+	+																	
n-C_6H_{13}	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±	−	±								
n-C_7H_{15}	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±	−	±	−	−	±
n-C_8H_{17}[a]																			−	−	−	−	−	−	−	−	±
C_3H_5	−	+	+	+	+	+	+	+	+	+																	
Cyclohexylpropyl alkyl acetic acids, $C_6H_{11}(CH_2)_3CH(COOH)R$. R =																											
C_2H_5	−	−	+	+	+	+	+	+	+																		
n-C_3H_7	−	−	±	+	+	+	+	+	+																		
n-C_4H_9	−	−	−	−	−	±	−	+	−	±																	
n-C_5H_{11}	−	−	−	−	−	−	±	−	−	−	−	−	−	−	−	−	−	±	+								
n-C_6H_{13}	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	±	±	±
n-C_7H_{15}	−	−	−	−	±	−	±	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±	−	±	±	±	±
C_4H_5	−	+	±	+	±	±	±	+	+	+																	
Cyclohexylbutyl alkyl acetic acids, $C_6H_{11}(CH_2)_4CH(COOH)R$. R =																											
C_2H_5	−	−	−	−	−	−	−	−	−	−	−	±	±	−	±	−	−	−	−	±	±	±	−	−	−	−	
n-C_3H_7	−	−	−	±	±	−	±	+	+	+	−	−	+	+	+	+	+	+	+									
n-C_4H_9	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	±	−	−	−
n-C_5H_{11}	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	±	±	−	−
n-C_6H_{13}	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±	+	−	±	±	−	+	−	±
C_5H_5	−	−	−	−	−	±	+	+	−	−	+	+	+	+	+	+	+	+	+								

[a] Not tested in dilutions under 190,000.

A maximum bactericidal effect is not reached until a certain sized side chain is present, for example, one containing ten, eleven or twelve carbon atoms. It is probable, therefore, that in acids of this type molecular weight plays an important role, perhaps by producing the proper physical properties in the compounds tested. It still remains to determine whether the side chain must be straight or whether it may be forked.

The allyl derivatives in each series of acids studied appeared to have essentially the same effect as the propyl derivatives. Subcultures were made with many of the acids. Results showed that in all instances they were bactericidal and not merely inhibitive.

The three series of acids were prepared in one of two ways. The β-cyclohexylethyl bromide, γ-cyclohexylpropyl bromide and the δ-cyclohexylbutyl bromide were condensed with the sodium derivatives of diethyl alkyl malonates to give the corresponding diethyl-β-cyclohexylethyl, γ-cyclohexylpropyl or δ-cyclohexylbutyl alkyl malonates. The di-substituted malonic esters were saponified with alcoholic potassium hydroxide

and the dibasic acids decomposed in the usual way to give the monobasic acids. The second procedure merely consisted in introducing into the malonic ester the ω-cyclohexylalkyl group first and the alkyl group second. Apparently there is very little choice in the procedures.

Isomeric acids with the carboxyl on the first and second carbon atoms from the cyclohexane ring will soon be completed.

The bacteriological work was carried out by Gerald H. Coleman and W. M. Stanley.

Experimental

β-Cyclohexylethyl Bromide, γ-Cyclohexylpropyl Bromide and δ-Cyclohexylbutyl Bromide.—These alkyl halides were prepared according to the method described by Hiers with Adams.[1g]

Alkyl Halides.—The alkyl halides were prepared from the corresponding alcohols. Of these alcohols, the *n*-amyl alcohol was produced from *n*-butyl magnesium bromide and formaldehyde,[3] *n*-heptyl alcohol[4] by the reduction of heptylic aldehyde with iron and acetic acid, *n*-octyl alcohol by the action of formaldehyde upon heptyl magnesium bromide,[3] and *n*-nonyl alcohol[4] either by the reduction of nonylic aldehyde with iron and acetic acid or by the condensation of *n*-heptyl magnesium bromide with ethylene oxide.[1g]

Diethyl-ω-Cyclohexylalkyl Alkyl Malonates.—These were prepared by the condensation of ω-cyclohexylalkyl bromides with the sodium derivatives of diethyl alkyl malonates, using the usual procedure. The yields were quite satisfactory though they could be improved somewhat by distilling off the alcohol (in an oil-bath at 110–130°) from the sodium diethyl alkyl malonate before adding the ω-cyclohexylalkyl bromides and refluxing the mixture for several hours.

ω-Cyclohexylalkyl Alkyl Malonic Acids.—These acids were prepared by adding the diethyl-ω-cyclohexylalkyl alkyl malonates to an excess of hot, saturated alcoholic potassium hydroxide solution. The mixture was heated for an hour on a water-bath under reflux and then evaporated to dryness, taking particular care that all of the alcohol was removed. The solid potassium salt was dissolved in a little water and the dibasic acid precipitated with hydrochloric acid and extracted with ether. The malonic acids were purified by crystallization from benzene or acetone. If the former was used the product frequently contained benzene of crystallization. Where the dibasic acids were not

TABLE II

DIETHYL-β-CYCLOHEXYLETHYL ALKYL MALONATES, $C_6H_{11}(CH_2)_2C(CO_2C_2H_5)_2R$

R =	B. p., °C.	n_D^{25}	d_4^{25}	Calcd., % C	Calcd., % H	Found, % C	Found, % H
C_2H_5	146–148 (2 mm.)	1.4502	0.9907	68.30	10.13	68.39	10.04
n-C_3H_7	153–156 (6 mm.)	1.4518	.9813	69.16	10.32	69.45	10.14
n-C_4H_9	144–147 (4 mm.)	1.4531	.9714	69.88	10.50	69.56	10.39
n-C_5H_{11}	174–176 (5 mm.)	1.4537	.9644	70.52	10.66	70.36	10.61
n-C_6H_{13}	188–191 (5 mm.)	1.4539	.9569	71.12	10.81	71.31	10.42
n-C_7H_{15}	171–173 (3 mm.)	1.4545	.9527	71.67	10.94	71.71	10.93
n-C_8H_{17}	213–216 (7 mm.)	1.4550	.9449	72.19	11.07	71.94	10.89
C_6H_5	142–145 (2 mm.)	1.4563	.9915	69.62	9.74	69.54	9.66

[3] For general procedure see "Organic Syntheses," John Wiley and Sons, Inc., New York City, 1926, Vol. 6, p. 22.

[4] *Ibid.*, p. 52.

readily handled they were decomposed directly to the monobasic acids without purification.

ω-Cyclohexylalkyl Alkyl Acetic Acids.—These were prepared by heating the malonic acids under reflux for two to three hours in an oil-bath at 20–30° above the melting point.

TABLE III

DIETHYL-γ-CYCLOHEXYLPROPYL ALKYL MALONATES, $C_6H_{11}(CH_2)_3C(CO_2C_2H_5)_2R$

R =	B. p., °C.	n_D^{25}	d_4^{25}	Calcd., % C	Calcd., % H	Found, % C	Found, % H
C_2H_5	149–151 (4 mm.)	1.4528	0.9797	69.17	10.33	69.01	10.43
n-C_3H_7	155–156 (4 mm.)	1.4531	.9743	69.87	10.51	69.81	10.53
n-C_4H_9	160–161 (4 mm.)	1.4534	.9620	70.53	10.66	70.46	10.70
n-C_5H_{11}	178–180 (4 mm.)	1.4549	.9603	71.13	10.81	71.05	10.72
n-C_6H_{13}	189–191 (4 mm.)	1.4551	.9501	71.68	10.95	71.71	10.98
n-C_7H_{15}	209–210 (5 mm.)	1.4554	.9471	72.18	11.07	71.74	11.18
C_3H_5	170–172 (5 mm.)	1.4569	.9837	70.30	9.94	70.10	9.88

TABLE IV

DIETHYL-δ-CYCLOHEXYLBUTYL ALKYL MALONATES, $C_6H_{11}(CH_2)_4C(CO_2C_2H_5)_2R$

R =	B. p., °C.	n_D^{25}	d_4^{25}	Calcd., % C	Calcd., % H	Found, % C	Found, % H
C_2H_5	165–167 (4 mm.)	1.4536	0.9704	69.87	10.50	69.61	10.24
n-C_3H_7	173–175 (4 mm.)	1.4538	.9695	70.52	10.66	70.59	10.43
n-C_4H_9	175–177 (4 mm.)	1.4546	.9563	71.12	10.81	71.02	10.44
n-C_5H_{11}	191–193 (5 mm.)	1.4559	.9530	71.67	10.92	71.43	10.79
n-C_6H_{13}	194–196 (4 mm.)	1.4572	.9514	72.19	11.07	72.40	10.86
C_3H_5	168–170 (4 mm.)	1.4565	.9742	70.94	10.13	70.83	10.09

TABLE V

β-CYCLOHEXYLETHYL ALKYL MALONIC ACIDS, $C_6H_{11}(CH_2)_2C(CO_2H)_2R$

R =	M. p., °C.	Calcd., mol. wt.	Found, neut. equiv.
C_2H_5	114–115	242.2	243.6
n-C_3H_7	132–133	256.2	258.8
n-C_4H_9	135–136	270.2	269.1
n-C_5H_{11}	125–126	284.2	285.6
n-C_8H_{17}	108–109	326.3	328.4
C_3H_5	95–96	254.2	253.1

TABLE VI

γ-CYCLOHEXYLPROPYL ALKYL MALONIC ACIDS, $C_6H_{11}(CH_2)_3C(CO_2H)_2R$

R =	M. p., °C.	Calcd., mol. wt.	Found, neut. equiv.
C_2H_5	143	256.2	254.8
n-C_3H_7	130	270.2	268.0
n-C_4H_9	138	284.2	281.1
n-C_5H_{11}	148	298.2	296.4
n-C_6H_{13}	134	312.2	312.4
n-C_7H_{15}	99	324.2	321.8

TABLE VII

δ-CYCLOHEXYLBUTYL ALKYL MALONIC ACIDS, $C_6H_{11}(CH_2)_4C(CO_2H)_2R$

R =	M. p., °C.	Calcd., mol. wt.	Found, neut. equiv.
C_2H_5	136	270.2	271.6
n-C_3H_7	140	284.2	286.8
n-C_4H_9	113	298.2	301.6
n-C_5H_{19}	64	312.2	316.3
C_8H_5	143	282.2	284.1

TABLE VIII

β-CYCLOHEXYLETHYL ALKYL ACETIC ACIDS, $C_6H_{11}(CH_2)_2CH(CO_2H)R$

R =	B. p., °C.	n_D^{25}	d_4^{25}	Calcd., mol. wt.	Found, neut. eq.	Calcd., % C	Calcd., % H	Found, % C	Found, % H
C_2H_5	121–124 (3 mm.)	1.4613	0.9619	198.2	199.0	72.66	11.19	72.52	11.41
n-C_3H_7	122–125 (2 mm.)	1.4623	.9486	212.2	213.2	73.51	11.41	73.42	11.46
n-C_4H_9	139–142 (4 mm.)	1.4624	.9410	226.2	228.0	74.22	11.58	74.02	11.43
n-C_5H_{11}	182–185 (5 mm.)	1.4626	.9350	240.2	242.9	74.93	11.74	75.14	11.76
n-C_6H_{13}	174–177 (2 mm.)	1.4628	.9283	254.2	258.0	75.53	11.89	75.21	11.66
n-C_7H_{15}	182–185 (2 mm.)	1.4631	.9222	268.3	266.0	76.04	12.02	75.73	11.86
n-C_8H_{17}	193–196 (4 mm.)	1.4640	.9193	282.3	281.8	76.51	12.14	76.86	11.89
C_3H_5	125–128 (2 mm.)	1.4672	.9714	210.2	208.0	74.22	10.55	74.16	11.44

TABLE IX

γ-CYCLOHEXYLPROPYL ALKYL ACETIC ACIDS, $C_6H_{11}(CH_2)_3CH(CO_2H)_2R$

R =	B. p., °C.	n_D^{25}	d_4^{25}	Calcd., mol. wt.	Found, neut. eq.	Calcd., % C	Calcd., % H	Found, % C	Found, % H
C_2H_5	146–147 (2 mm.)	1.4622	0.9509	212.2	210.8	73.51	11.40	73.49	11.30
n-C_3H_7	148–150 (2 mm.)	1.4627	.9419	226.2	225.7	74.26	11.58	74.20	11.47
n-C_4H_9	153–154 (2 mm.)	1.4630	.9317	240.2	242.1	74.93	11.75	74.89	11.81
n-C_5H_{11}	188–192 (5 mm.)	1.4634	.9266	254.2	253.4	75.53	11.89	75.25	12.01
n-C_6H_{13}	208–211 (8 mm.)	1.4638	.9221	268.2	269.1	76.09	12.04	76.01	12.07
n-C_7H_{15}	199–203 (2 mm.)	1.4642	.9137	282.2	279.0	76.56	12.15	76.40	12.07
C_3H_5	147–150 (2 mm.)	1.4708	.9552	224.2	225.0	74.93	10.78	74.78	10.71

TABLE X

δ-CYCLOHEXYLBUTYL ALKYL ACETIC ACIDS, $C_6H_{11}(CH_2)_4CH(CO_2H)R$

R =	B. p., °C.	n_D^{25}	d_4^{25}	Calcd., mol. wt.	Found, neut. eq.	Calcd., % C	Calcd., % H	Found, % C	Found, % H
C_2H_5	173–175 (3 mm.)	1.4622	0.9447	226.2	227.5	74.27	11.55	74.20	11.52
n-C_3H_7	156–158 (1 mm.)	1.4627	.9408	240.2	241.2	74.93	11.72	74.75	11.53
n-C_4H_9	178–180 (4 mm.)	1.4631	.9300	254.2	254.7	75.53	11.89	75.56	11.74
n-C_5H_{11}	207–209 (8 mm.)	1.4633	.9254	268.2	269.0	76.06	12.03	75.86	12.12
n-C_6H_{13}	187–189 (1 mm.)	1.4638	.9191	282.2	282.8	76.54	12.14	76.43	12.00
C_3H_5	174–176 (6 mm.)	1.4687	.9531	238.2	239.8	75.56	11.00	75.45	10.79

Summary

1. Three series of acids of the general formulas $C_6H_{11}(CH_2)_2CH(CO_2H)R$, $C_6H_{11}(CH_2)_3CH(CO_2H)R$ and $C_6H_{11}(CH_2)_4CH(CO_2H)R$ were prepared in which the R group was varied so that isomeric acids containing eight to twelve carbons, inclusive, in the side chain were produced. Some acids of lower molecular weight were also synthesized.

2. With increase in molecular weight the bactericidal effect toward acid-fast bacteria increased markedly until the higher molecular weight

compounds in the form of their sodium salts were much more effective than the sodium salts of pure chaulmoogric or hydnocarpic acids or the sodium salts of any of the mixed acids from natural oils containing chaulmoogric or hydnocarpic acids.

URBANA, ILLINOIS

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF ILLINOIS]

CERTAIN Δ^2-CYCLOPENTENYL ALKYL ACETIC ACIDS AND THEIR ACTION TOWARD B. LEPRAE. IX[1]

BY JAMES A. ARVIN[2] WITH ROGER ADAMS

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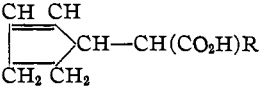
The effect of the size of the side chain on the bactericidal character of various acids containing the cyclohexyl group was shown in a research described in the preceding paper.[1] The number of carbon atoms present apparently played such an important role in these compounds that it seemed probable that it would play just as important a one certainly in other series of acids, and probably in other classes of compounds now being studied in this same field.

Perkins[3] prepared Δ^2-cyclopentenyl alkyl acetic acids in which the alkyl group was ethyl, *n*-propyl, *n*-butyl and allyl, and reported that some of these acids showed sufficient bactericidal action toward *B. Leprae* to warrant clinical testing. Judging from the results on cyclic acids in this Laboratory, by far the most effective compounds in this series should be those in which the alkyl group is octyl, or nonyl, or of even higher molecular weight. Since these substances have not previously been made, a series of Δ^2-cyclopentenyl alkyl acetic acids has been produced and tested in which the alkyl group varies from *n*-amyl to *n*-nonyl (I).



I

The results were exactly those predicted. The bactericidal action increased very rapidly from *n*-amyl to the *n*-nonyl, the *n*-hexyl killing in dilutions of 1:10,000, but the higher molecular weight compounds in very much greater dilutions, 1:150,000 in the *n*-nonyl (Table I).

The compounds were prepared by condensing the sodium derivative of diethyl-Δ^2-cyclopentenyl malonate with various alkyl halides, preferably in the absence of the alcohol, so that a higher temperature might be reached

[1] Paper VIII in this series, THIS JOURNAL, **49**, 2934 (1927).

[2] This communication is an abstract of a portion of the thesis submitted by James A. Arvin in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Chemistry at the University of Illinois.

[3] Perkins and Cruz, THIS JOURNAL, **49**, 517 (1927).