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The Structure of Viscosin, A Peptide Antibiotic. I. Syntheses of D- and L-3-Hydroxyacyl-L-leucine Hydrazides Related to Viscosin[1]

Minoru Hiramoto, Kozo Okada, Sotoo Nagai and Hiroshi Kawamoto

Faculty of Pharmaceutical Sciences, Kanazawa University[2]

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Sixteen D- and L-3-hydroxyacyl-L-leucine hydrazides related to viscosin have been synthesized. Condensation of DL-3-hydroxy fatty acids with L-leucine methyl ester by the azide method or the active ester method gave DL-3-hydroxyacyl-L-leucine methyl esters, which were then converted to their hydrazides. Each of the racemic hydrazides could readily be resolved into the respective optically active diastereoisomers by means of fractional crystallization using alcohols.

All of the D- and L-3-hydroxyacyl-L-leucine hydrazides prepared showed antimicrobial activity against four kinds of microorganisms only comparable to or less than that of the saturated straight chain fatty acids of corresponding carbon numbers.

Viscosin is an acidic antibiotic first isolated from the culture fluid of *Pseudomonas viscosa* by Kochi,[3] which was reported to be active to tubercle bacillus and certain viruses.[4] Ohno and his coworkers[5] proposed the structure (Ia) as a peptidolipid containing 3-hydroxy acid at the N-terminus of hexapeptide.

D-$CH_3(CH_2)_6CH(OH)CH_2CO$-L-Leu-Gly-L-Ser-R-L-Thr-L-Leu-OH Ia, b

L-$CH_3(CH_2)_6CH(OH)CH_2CO$-L-Leu-Gly-L-Ser-R-L-Thr-L-Leu-OH IIa, b

a: R=D-Val b: R=L-Val

Hitomi, *et al.*[6] have prepared several analogs of Ia, acyl-L-leucyl-glycyl-L-seryl-D-valyl-L-threonyl-L-leucine (acyl: hexanoyl, octanoyl, decanoyl, myristoyl, palmitoyl and stearoyl) and shown that some of the analogs had antituberculous activity at a level of 100 μg/ml of minimum inhibitory concentration.

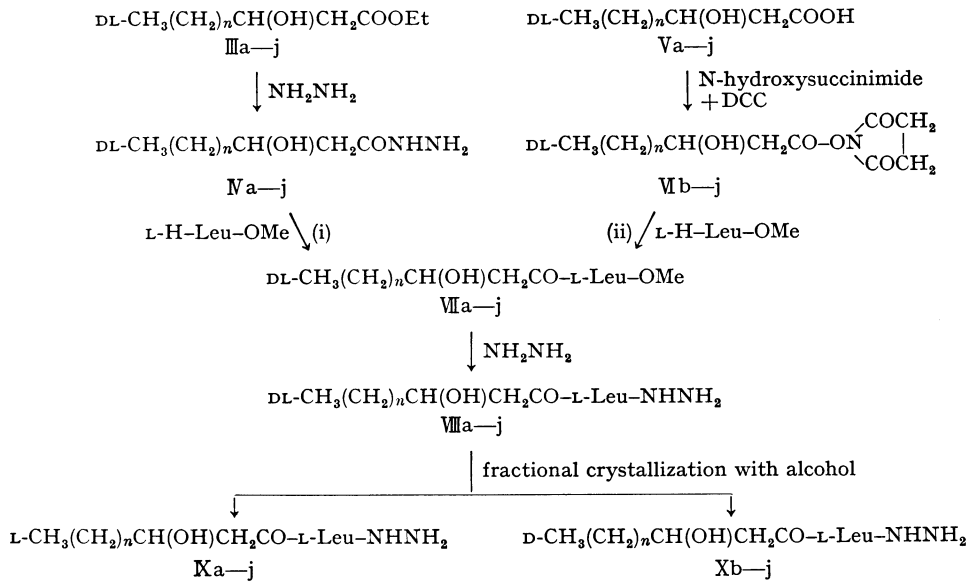
Furthermore, the fact that some naturally occurring depsipeptides such as fortuitine,[7] esperine,[8] surfactin[9] and the peptidolipids from *Mycobacterium johnei* and *Nocardia asteroides*[10] have been isolated only as a mixture with closely related compounds, usually with a few kinds of acyl homologs, suggests possible heterogeneity of viscosin preparation. In addition, no clear evidence for D-configuration of valine in viscosin molecule has been presented up to date.

1) A part of this study has been reported in *Biochem. Biophys. Res. Commun.*, **35**, 702 (1969).
2) Location: *Takara-machi, 13, Kanazawa.*
3) M. Kochi, Japan Patent, 193872 (1951).
4) M. Kochi, V. Groupé, L.H. Pugh and D. Weiss, *Bact. Rev.*, **1951**, 291; V. Groupé, L.H. Pugh, D. Weiss and M. Kochi, *Proc. Soc. Exp. Biol. Med.*, **78**, 354 (1951).
5) T. Ohno, S. Tajima and K. Toki, *Nippon Nogeikagaku Kaishi*, **27**, 665 (1953); K. Toki and T. Ohno, *ibid.*, **29**, 370 (1955).
6) H. Hitomi, M. Uchiyama and K. Fukuda, *Yakugaku Zasshi*, **88**, 299 (1969).
7) M. Barber, P. Jolles, E. Vilkas and E. Lederer, *Biochem. Biophys. Res. Commun.*, **18**, 469 (1965).
8) D.W. Thomas and T. Ito, *Tetrahedron*, **25**, 1985 (1969).
9) A. Kakinuma, H. Sugino, M. Isono, G. Tamura and K. Arima, *Agr. Biol. Chem.* (Tokyo), **33**, 973 (1969).
10) G. Laneelle, J. Asselineau, W.A. Wolstenholme and E. Lederer, *Bull. Soc. Chim. France*, **1965**, 2133; M. Guinand, M.J. Vacheron, G. Michel, B.C. Das and E. Lederer, *Tetrahedron, Suppl.* **7**, 271 (1966); M. Guinand, G. Michel, B.C. Das and E. Lederer, *Vietnamica Chim. Acta*, **1966**, 37.

Ambiguities on the proposed structure (Ia) for viscosin arising from the above points prompted us to undertake synthetic study of a group of 3-hydroxyacylhexapeptides including Ia, b and their stereoisomers (IIa, b).

We found it convenient for our purpose to use L- or D-3-hydroxyacyl-L-leucine hydrazides (IX or X) as synthetic intermediates, since the optically active compounds could be easily obtained from their diastereoisomeric mixtures (VIII) by fractional crystallization and directly utilized for the subsequent peptide coupling reaction *via* the azides.

The present communication concerns the synthesis of these hydrazides IX and X. The synthetic routes are outlined in Chart 1.



a, $n=2$; b, $n=4$; c, $n=5$; d, $n=6$; e, $n=7$; f, $n=8$; g, $n=9$; h, $n=10$; i, $n=12$; j, $n=14$

Chart 1

The intermediates, DL-3-hydroxyacyl-L-leucine methyl esters (VII) were prepared by the following two routes. (i) DL-3-Hydroxy fatty acid ethyl ester (III), prepared by either

TABLE I. DL-$CH_3(CH_2)_nCH(OH)CH_2CONHNH_2$ IV

	n	Recryst. solvt.	Yield[a] (%)	mp (°C)	Formula	Analysis (%) Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
IVa	2	EtOH–petr.ether	28	104—105	$C_6H_{14}O_2N_2$	49.30	9.65	19.17	49.58	9.55	19.26
IVb	4	EtOH	36	105—107	$C_8H_{18}O_2N_2$	55.14	10.41	16.08	55.00	10.16	16.27
IVc	5	EtOH	42	111—112	$C_9H_{20}O_2N_2$	57.41	10.71	14.88	57.62	10.88	14.50
IVd	6	EtOH	40	109—110	$C_{10}H_{22}O_2N_2$	59.37	10.96	13.85	59.49	11.14	13.81
IVe	7	EtOH	41	114—115	$C_{11}H_{24}O_2N_2$	61.07	11.18	12.95	61.05	11.14	13.30
IVf	8	EtOH–ether	50	115—117	$C_{12}H_{26}O_2N_2$	62.57	11.38	12.16	62.30	11.40	12.04
IVg	9	EtOH	45	118—120	$C_{13}H_{28}O_2N_2$	63.89	11.55	11.46	63.66	11.43	11.37
IVh	10	EtOH–petr.ether	45	118—119	$C_{14}H_{30}O_2N_2$	65.07	11.70	10.84	65.27	11.76	10.95
IVi	12	EtOH	47	121—122	$C_{16}H_{34}O_2N_2$	67.09	11.96	9.78	67.37	11.66	9.95
IVj	14	EtOH	48	123[b]	$C_{18}H_{38}O_2N_2$	68.74	12.18	8.91	68.94	12.08	9.30

a) IVi and IVj; from the corresponding 3-keto acids, the others; from the corresponding aldehydes
b) lit.[11] mp 123—124° (decomp.)

11) F. Adickes and G. Anderson, *Ann.*, **555**, 46 (1944); H. Thaler and G. Geist, *Biochem. Z.*, **302**, 374 (1939).

Reformatsky reaction[11] or sodium borohydride reduction of the corresponding 3-keto acid ester, was converted to the hydrazide (IV) (Table I), which was coupled with L-leucine methyl ester by means of the azide method to give oily product (VII). (ii) III was saponified to the corresponding free acid (V), which was converted into the N-hydroxysuccinimide ester (VI)[12] (Table III), and VI was coupled with L-leucine methyl ester to yield VII.

VII was then converted, without purification, into the corresponding hydrazide, DL-3-hydroxyacyl-L-leucine hydrazide (VIII). The resolution of VIII into the optically active diastereoisomers (IX and X) could be readily accomplished by fractional crystallization using alcohols such as MeOH (for VIIIb—g), EtOH (for VIIIh—j) or iso-PrOH (for VIIIa).

TABLE II. Ephedrine(Eph.) Salt of L- or D-$CH_3(CH_2)_nCH(OH)CH_2COOH$ V'b, c, f, h or V"b, c, f, h[a]

Salt	Yield (%)	mp (°C)	$[\alpha]_D$ (c in $CHCl_3$, °C)	Formula	Analysis (%) Calcd. C	H	N	Found C	H	N
V'b-(−)Eph.	13	100 —101	+36.4 (5, 18)	$C_{18}H_{31}O_4N$	66.43	9.60	4.30	66.77	9.54	4.38
V"b-(+)Eph.	13	101 —102	−36.8 (5, 18)		66.43	9.60	4.30	66.63	9.53	4.56
V'c-(+)Eph.	26	103 —104.5	− 7.0 (2, 22)	$C_{19}H_{33}O_4N$	67.22	9.80	4.13	67.59	9.83	4.59
V"c-(−)Eph.	23	103 —104.5	+ 7.0 (2, 22)		67.22	9.80	4.13	67.13	9.73	4.49
V'f-(+)Eph.	34	94 — 94.5	− 8.8 (2, 18)	$C_{22}H_{39}O_4N$	69.25	10.30	3.67	69.10	10.35	3.75
V"f-(−)Eph.	32	94 — 94.5	+ 8.8 (2, 18)		69.25	10.30	3.67	69.45	10.46	4.12
V'h-(−)Eph.	34	96 — 97.5	+30.0 (1, 16)	$C_{24}H_{43}O_4N$	70.37	10.58	3.42	70.65	10.49	3.72
V"h-(+)Eph.	39	96.5— 97.5	−31.1 (1, 16)		70.37	10.58	3.42	70.69	10.61	3.33

a) b: n= 4; c: n=5; f: n=8; h: n=10

TABLE III. DL-, L- or D-$CH_3(CH_2)_nCH(OH)CH_2COO$-N$<^{CO-CH_2}_{CO-CH_2}$ VI, VI' or VI"

	n	Yield (%)	mp[a] (°C)	$[\alpha]_D$ (c, solv., °C)	Formula	Analysis (%) Calcd. C	H	N	Found C	H	N
VIb	4	77	59 — 61		$C_{12}H_{19}O_5N$	56.02	7.44	5.44	55.81	7.36	5.93
VI'b	4	61	74 — 75	+14.0 (2, $CHCl_3$, 14)					55.40	7.30	5.84
VI"b	4	72	73.5— 74.5	−14.0 (2, $CHCl_3$, 14)					55.51	7.21	5.85
VIc	5	74	66 — 68		$C_{13}H_{21}O_5N$	57.55	7.80	5.16	57.64	7.96	5.18
VI'c	5	80	84 — 85.5	+14.1 (2, $CHCl_3$, 16)					57.85	7.83	5.44
VI"c	5	79	86 — 86.5	−14.1 (2, $CHCl_3$, 16)					57.90	7.76	5.23
VId	6	88	78 — 78.5		$C_{14}H_{23}O_5N$	58.93	8.13	4.91	59.11	8.11	5.34
VI'd	6	62	91 — 92	− 3.1 (1, AcOEt, 18)					59.08	8.06	5.03
VI"d	6	83	93 — 94	+ 3.0 (1, AcOEt, 18.5)					58.71	8.06	4.91
VIe	7	72	79 — 81		$C_{15}H_{25}O_5N$	60.18	8.42	4.61	60.09	8.56	4.32
VIf	8	90	86 — 88		$C_{16}H_{27}O_5N$	61.32	8.68	4.47	61.88	8.74	4.66
VI'f	8	89	99 —100	+11.9 (1.8, $CHCl_3$, 18)					60.85	8.54	4.59
VI"f	8	82	98 — 99.5	−12.7 (2, $CHCl_3$, 18)					60.94	8.59	4.48
VIg	9	86	88.5— 89		$C_{17}H_{29}O_5N$	62.36	8.93	4.28	62.95	9.06	4.15
VIh	10	72	90.5— 91.5		$C_{18}H_{31}O_5N$	63.31	9.15	4.10	62.77	9.01	4.78
VI'h	10	68	104.5—105	+12.1 (2.1, $CHCl_3$, 15)					63.04	9.08	4.46
VI"h	10	76	104.5—105	−12.0 (1.8, $CHCl_3$, 15)					63.51	9.09	4.38
VIi	12	67	95.5— 96.5		$C_{20}H_{35}O_5N$	65.01	9.55	3.79	65.75	9.72	4.10
VIj	14	58	98 — 99.5		$C_{22}H_{39}O_5N$	66.46	9.89	3.52	66.27	9.80	3.92

a) VIb—h, VI'b—h and VI"b—h: recrystallized from AcOEt-petr. ether, VIi and VIj: recrystallized from AcOEt

12) G.W. Anderson, J.S. Zimmerman and F.M. Callahan, *J. Am. Chem. Soc.*, **86**, 1839 (1964).

TABLE IV. L-$CH_3(CH_2)_nCH(OH)CH_2CO$-L-Leu-$NHNH_2$ IX

	n	Method[a]	Recryst. solv.	Yield[b] (%)	mp (°C)	$[\alpha]_D$ (c, solv., °C)	Formula	Analysis (%) Calcd. / Found C	H	N
IXa	2	A-(i)	iso-PrOH	27	188 —189	−13.4 (2, AcOH, 20)	$C_{12}H_{25}O_3N_3$	55.76 / 55.90	9.68 / 9.63	16.23 / 16.36
IXb	4	A-(ii)	MeOH	26	180 —181[c]	−15.9 (2, AcOH, 20)	$C_{14}H_{29}O_3N_3$	58.50 / 58.34	10.17 / 10.08	14.62 / 14.31
		B	MeOH	77	180 —181	−16.0 (2, AcOH, 19)		58.50 / 58.86	10.17 / 10.15	14.62 / 14.27
IXc	5	A-(ii)	MeOH	12	166 —168[c]	−22.5 (2, AcOH, 18)	$C_{15}H_{31}O_3N_3$	59.77 / 59.41	10.37 / 10.25	13.94 / 13.61
		B	MeOH	70	168 —169	−24.0 (2 ,AcOH, 16)		59.77 / 59.91	10.37 / 10.28	13.94 / 14.17
IXd	6	A-(i)	MeOH	33	165 —166[c]	−16.8 (1 ,AcOH, 18)	$C_{16}H_{33}O_3N_3$	60.92 / 60.92	10.54 / 10.25	13.32 / 13.41
		A-(ii)	MeOH	24	165 —167	−17.2 (5 ,AcOH, 15)		60.92 / 60.82	10.54 / 10.49	13.32 / 13.38
		B	MeOH	74	165.5—166.5	−16.7 (2.2, AcOH, 16)		60.92 / 61.02	10.54 / 10.46	13.32 / 13.27
IXe	7	A-(ii)	MeOH	20	159 —161	−21.0 (2, AcOH, 17)	$C_{17}H_{35}O_3N_3$	61.97 / 62.04	10.71 / 10.58	12.76 / 12.56
IXf	8	A-(ii)	MeOH	36	160 —162[c]	−17.4 (2 ,AcOH, 16)	$C_{18}H_{37}O_3N_3$	62.93 / 62.79	10.86 / 10.73	12.23 / 12.24
		B	MeOH	86	162 —164	−16.0 (2, AcOH, 16)		62.93 / 63.27	10.86 / 10.92	12.23 / 12.46
IXg	9	A-(ii)	MeOH	17	157 —159	−19.5 (2, AcOH, 18)	$C_{19}H_{39}O_3N_3$	63.82 / 63.64	11.10 / 11.20	11.75 / 11.43
IXh	10	A-(i)	EtOH	32	156 —159[c]	−15.0 (1, AcOH, 22)	$C_{20}H_{41}O_3N_3$	64.65 / 64.43	11.12 / 11.07	11.31 / 11.22
		A-(ii)	EtOH	36	153 —155	−14.0 (1, AcOH, 22)		64.65 / 64.80	11.12 / 11.17	11.31 / 11.15
		B	MeOH	86	159 —161	−15.6 (1.2, AcOH, 18)		64.65 / 64.85	11.12 / 11.05	11.31 / 11.55
IXi	12	A-(i)	EtOH	16	151 —152	−17.2 (2, HCOOH, 15)	$C_{22}H_{45}O_3N_3$	66.12 / 66.31	11.35 / 11.39	10.52 / 10.59
IXj	14	A-(i)	EtOH	24	144 —147	−14.0 (0.5, EtOH, 29)	$C_{24}H_{40}O_3N_3$	67.40 / 67.29	11.55 / 11.52	9.83 / 9.79

a) A: Prepared by fractional crystallization of VIII, which was obtained from VII prepared by (i) azide method or (ii) active ester method. B: prepared from VI'.
b) from IV, VI or VI'
c) undepressed on admixture with a sample prepared by method B

In order to assess optical purity of the products thus obtained, IXb—d, f, h and Xb—d, f, h were also synthesized by another route. Optically active 3-hydroxy fatty acids were prepared by the cinchonidine[13] or ephedrine resolution of the respective racemic acids (Vb—d, f, h) (Table II) and then converted to the corresponding N-hydroxysuccinimide esters (VI' and VI'') (Table III), which were then reacted with L-leucine methyl ester followed by hydrazinolysis to afford IXb—d,f,h and Xb—d,f,h respectively. The preparations of each compound obtained by the two routes were identical in mp and optical rotation. From these data, it is quite probable that the fractional crystallization of the diastereoisomeric mixture (VIII) described above gave satisfactory results.

The yields, physical constants and elemental analyses of the synthesized N-acyl-L-leucine hydrazides are given in Table IV and V.

13) N.J. Cartwright, *Biochem. J.*, **67**, 663 (1957).

TABLE V. D-$CH_3(CH_2)_nCH(OH)CH_2CO$-L-Leu-$NHNH_2$ X

	n	Method[a]	Recryst. solv.	Yield[b] (%)	mp (°C)	$[\alpha]_D$ (c,solv., °C)	Formula	Analysis (%) Calcd. Found C	H	N
Xb	4	A-(ii)	MeOH–ether	7	145 —150[c]	−41.5 (2, AcOH, 15)	$C_{14}H_{32}O_3N_3$	58.50	10.17	14.62
								58.52	10.52	14.39
		B	MeOH–ether	40	147 —149	−40.0 (2, AcOH, 19)		58.50	10.17	14.62
								58.65	10.07	14.40
Xc	5	A-(ii)	MeOH–benzene	14	142 —146[c]	−44.5 (2, AcOH, 18)	$C_{15}H_{31}O_3N_3$	59.77	10.37	13.94
								59.87	10.32	13.77
		B	MeOH–ether	47	145 —147	−46.0 (2.7, AcOH, 19)		59.77	10.37	13.94
								60.05	10.37	14.06
Xd	6	A-(ii)	MeOH–ether	21	144 —145[c]	−36.0 (5, AcOH, 13)	$C_{16}H_{33}O_3N_3$	60.92	10.54	13.32
								60.80	10.54	13.49
		B	MeOH–ether	68	146 —147	−34.0 (2, AcOH, 15)		60.92	10.54	13.32
								60.64	10.40	13.13
Xe	7	A-(ii)	MeOH–benzene	23	130 —131.5	−40.5 (2, AcOH, 18)	$C_{17}H_{35}O_3N_3$	61.97	10.71	12.76
								61.71	10.70	12.72
Xf	8	A-(ii)	MeOH–ether	26	126 —127	−36.5 (5, AcOH, 19)	$C_{18}H_{37}O_3N_3$	62.93	10.86	12.23
								62.75	10.78	11.93
		B	MeOH–ether	54	127.5—130	−36.8 (1.4, AcOH, 16)		62.93	10.86	12.23
								62.80	10.86	12.41
Xg	9	A-(ii)	MeOH–benzene	21	126 —130	−31.0 (2 ,AcOH, 18)	$C_{19}H_{39}O_3N_3$	63.83	11.10	11.75
								63.54	10.98	11.37
Xh	10	A-(i)	MeOH–ether	24	117 —122[c]	−26.0 (1, AcOH, 15)	$C_{20}H_{41}O_3N_3$	64.65	11.12	11.31
								64.57	11.08	11.27
		B	MeOH–ether	84	121 —123	−28.0 (1, AcOH, 18)		64.65	11.12	11.31
								64.70	11.15	11.46
Xi	12	A-(i)	benzene–ligroin	15	100 —105	−28.0 (2, HCOOH, 15)	$C_{22}H_{45}O_3N_3$	66.12	11.35	10.52
								66.09	11.26	10.84

a) A: Prepared by fractional crystallization of VIII, which was obtained from VII prepared by (i) azide method or (ii) active ester method. B: prepaerd from VI"
b) from IV, VI or VI"
c) undepressed on admixture with a sample prepared by method B

TABLE VI. Antibacterial Activity of DL-3-Hydroxy Fatty Acids (V), Their Hydrazides (IV) and L- and D-3-Hydroxyacyl-L-leucine Hydrazides (IX and X)[a] (Minimum Inhibitory Concentration, μg/ml)

Acyl carbon number	*E. coli*					*St. aureus*					*Candida albicans*				
	IV_{b-j}	V_{b-j}	IX_{a-j}	X_{b-i}	XI_{a-j}[b]	IV_{b-j}	V_{b-j}	IX_{a-j}	X_{b-i}	XI_{a-j}[b]	IV_{b-j}	V_{b-j}	IX_{a-j}	X_{b-i}	XI_{a-j}[b]
C_6			200		200			>200		200			200		100
C_8	200	200	200	200	200	>200	>200	>200	200	200	200	200	200	200	100
C_9	200	200	200	100	100	>200	>200	200	200	200	200	200	100	100	50
C_{10}	200	200	200	200	100	>200	>200	>200	200	200	200	200	200	200	50
C_{11}	200	200	200	100	200	>200	>200	200	200	200	200	>200	100	100	100
C_{12}	>200	200	200	200	100	>200	>200	>200	200	200	>200	>200	>200	100	100
C_{13}	>200	>200	100	100	100	>200	>200	200	200	200	>200	>200	100	50	100
C_{14}	>200	>200	200	200	100	>200	>200	>200	200	200	>200	>200	>200	100	100
C_{16}	>200	>200	200	>200	100	>200	>200	>200	200	200	>200	>200	200	>200	100
C_{18}	>200	>200	200		100	>200	>200	200		200	>200	>200	>200		100

a) Any of compounds with acyl carbon number C_8, C_{10} and C_{12} (IVb, d, f, Vb, d, f, IXb, d, f and Xb, d, f) showed no activity against *Mycobacterium tuberculosis* even at a concentration of 50 μg/ml of the assay medium, while INAH as a standard exhibited at 0.1 μg/ml minimum inhibitory concentration. The antituberclous activity of compounds other than the above could not be examined, owing to their insolubility in required solvents.
b) XI: saturated *n*-fatty acids

The antibacterial activities of DL-3-hydroxy fatty acids (V), their hydrazides (IV), and D- and L-3-hydroxyacyl-L-leucine hydrazides (IX and X) were examined with four kinds of microorganisms, and the activities were compared with those of saturated *n*-fatty acids. As shown in Table VI, most of the synthesized compounds exhibited antimicrobial activities only comparable to or less than those of the saturated *n*-fatty acids of the corresponding carbon numbers against any of the microorganisms tested.

Experimental[14]

DL-3-Hydroxy Fatty Acid Hydrazides (IVa—j) (Table I)——a) DL-3-Hydroxy Fatty Acid Ethyl Esters (IIIa—j): IIIa—h were prepared according to the literature,[11] and IIIi and IIIj were obtained by $NaBH_4$ reduction of the corresponding 3-oxo fatty acid esters as follows: to a solution of ethyl 3-oxooctadecanoate[15] (9.8 g, 0.03 mole) in EtOH (400 ml) was added dropwise $NaBH_4$ (0.015 mole) in H_2O (12 ml) with vigorous stirring at 20°. After stirring was continued for an additional 10 min, conc. NH_3 solution (4.3 ml) was added to the reaction mixture, and the solution was allowed to stand at room temperature for 1 hr. The reaction mixture was then poured into H_2O (60 ml), which was evaporated *in vacuo* to dryness. The resulting solid residue was extracted with ether (500 ml). The extract was washed successively with H_2O, 5% HCl and H_2O, dried over $MgSO_4$ and evaporated *in vacuo* to yield a crystalline residue, which was recrystallized from EtOH to give ethyl DL-3-hydroxyoctadecanoate (IIIj): Yield 5.8 g (59%), mp 46° (lit.[15] mp 46°).

Similarly, from ethyl 3-oxohexadecanoate[15] (8.9 g, 0.03 mole), was obtained ethyl DL-3-hydroxyhexadecanoate (IIIi) as an oily product: Yield 5.5 g (62%).

All the esters thus obtained were used without further purification.

b) DL-3-Hydroxy Fatty Acid Hydrazides (IVa—j): To a solution of III (0.05 mole) in EtOH was added hydrazine hydrate (0.2 mole) and the reaction mixture was refluxed for 2 hr. After cooling, the resulting crystal was collected by filtration and recrystallized from suitable solvent shown in Table I to give the hydrazide (IV) as colorless crystal.

DL-, L-, or D-3-Hydroxy Fatty Acid N-Hydroxysuccinimide Esters (VIb—j, VI'b—d,f,h or VI"b—d, f,h) (Table III)——a) DL-, L- or D-3-Hydroxy Fatty Acids (Vb—j, V'b—d,f,h or V"b—d,f,h): The racemic acids (Vb—j) were obtained from the corresponding esters (IIIb—j) by the usual saponification procedure. Their melting points were identical with those in the literature.[11,15] L- or D-3-Hydroxydecanoic acid (V'd or V"d) was prepared by cinchonidine resolution of Vd according to Cartwright.[13] V'd: mp 47—48°, $[\alpha]_D^{21}$+16.0° (c=1, $CHCl_3$) [lit.[13] mp 45—47°, $[\alpha]_D^{20}$+18±2° (c=1, $CHCl_3$)]. V"d: mp 46—47.5°, $[\alpha]_D^{21.5}$ −20.0° (c=1, $CHCl_3$) [lit.[13] mp 46—48°, $[\alpha]_D^{20}$−17.5±2° (c=1, $CHCl_3$)]. *p*-Bromophenacyl ester of V"d: mp 107°, $[\alpha]_D^{16.5}$−8.5 (c=2, benzene) (lit.[13] mp 105—106°). V'b,c,f,h and V"b,c,f,h were obtained by ephedrine resolution of the corresponding racemic acids according to the following general procedure: to a solution of DL-3-hydroxyfatty acid (0.018 mole) in ether (200 ml) was added (−)- or (+)-ephedrine $1/2H_2O$[16] (0.018 mole) and the solution was allowed to stand at room temperature for 1 or 2 days. The precipitate that formed was collected, washed with cold ether and recrystallized twice from ether and once from AcOEt to give scales of (−)- or (+)-ephedrine salt of optically active acid (Table II). The salt (3 mmole) thus obtained was suspended in AcOEt (120 ml) and treated with a slight excess of 2 N HCl, and the organic layer was washed with H_2O, dried over Na_2SO_4 and evaporated to dryness *in vacuo* to give a crystalline material. Recrystallization from petr. ether afforded almost theoretical yield of pure acid; L-3-hydroxyoctanoic acid (V'b): mp 20—22°, $[\alpha]_D^{18}$+23.0° (c=2, $CHCl_3$). *Anal.* Calcd. for $C_8H_{16}O_3$: C, 59.98; H, 10.07. Found: C, 59.55; H, 10.24. Hydrazide of V'b (prepared by treatment of V'b with CH_2N_2 followed by hydrazinolysis): mp 129—129.5°, $[\alpha]_D^{18}$+11.7° (c=1, H_2O) [lit.,[17] mp 127—128°, $[\alpha]_D$+12° (c=1.1, H_2O)]; the D-isomer (V"b): mp 20—22°, $[\alpha]_D^{18}$−23.0° (c=2, $CHCl_3$). *Anal.* Found: C, 60.29; H, 10.20. [lit.,[17] $[\alpha]_D^{20}$−21.0° (c=1.9, $CHCl_3$)]; Hydrazide of V"b: mp 129—129.5°, $[\alpha]_D^{18}$−11.4° (c=1.1, H_2O) [lit.,[17] mp 127.5—128°, $[\alpha]_D$−11.4° (c=1, H_2O)]; L-3-hydroxynonanoic acid (V'c): mp 51—52°, $[\alpha]_D^{22}$+22.0° (c=5, $CHCl_3$) [lit.,[18] mp 50—51°, $[\alpha]_D^{20}$+19.8° (c=5, $CHCl_3$)]: the D-isomer (V"c): mp 51—52°, $[\alpha]_D^{22}$−22.6° (c=2, $CHCl_3$). *Anal.* Calcd. for $C_9H_{18}O_3$: C, 62.04; H, 10.41. Found: C, 62.04; H, 10.46; L-3-hydroxydodecanoic acid (V'f): mp 62.5—63.5°, $[\alpha]_D^{19}$+15.1° (c=1.6, $CHCl_3$) [lit.,[19] mp 62.2—63.2°, $[\alpha]_D^{25}$+15.6±1° (c=2.1, $CHCl_3$)]; the D-isomer (V"f): mp 62.5—63°, $[\alpha]_D^{19}$−15.9° (c=1.5, $CHCl_3$) [lit.,[19] mp 62.5—63°, $[\alpha]_D^{25}$−15.2±1° (c=1.6,

14) All melting points are uncorrected. Optical rotations were determined on a Jasco DIP-SL polarimeter.
15) P.A. Levene and H.L. Haller, *J. Biol. Chem.*, **63**, 670 (1925).
16) Prepared from (+) or (−) -ephedrine HCl (Fuji Pharmaceutical Industries Co.) in the manner described by K. Oki, K. Suzuki, S. Tuchida, T. Saito, and H. Kotake [K. Oki, K. Suzuki, S. Tuchida, T. Saito and H. Kotake, *Bull. Chem. Soc., Japan*, **43**, 2554 (1970)].
17) R.U. Lemieux, *Can. J. Chem.*, **29**, 415 (1951); R.U. Lemieux and J. Giguere, *ibid.*, **29**, 678 (1951).
18) K. Serck-Hansen, *Chem. Ind.* (London), **1958**, 1554.
19) L.C. Vining and W.A. Taber, *Can. J. Chem.*, **40**, 1579 (1962).

$CHCl_3$)]; L-3-hydroxytetradecanoic acid (V'h): mp 72—73.5°, $[\alpha]_D^{25}$+10.6° (c=1.0, pyridine) [lit.,[20] mp 71—73°, $[\alpha]_D^{25}$+6.5° (c=1.5, pyridine), this contained *ca.* 15% of the D-acid]; the D-isomer (V"h): mp 73.5—74.5°, $[\alpha]_D^{25}$−10.4° (c=1.0, pyridine) [lit.[20] mp 71—73°, $[\alpha]_D^{25}$−9.4° (c=1.9, pyridine)].

b) DL-, L- or D-3-Hydtxy Fatty Acid N-Hydroxysuccinimide Esters (VIb—j, VI'b—d,f,h or VI"b—d, f,h): To a solution of DL-3-hydroxyfatty acid (V) (0.05 mole) and N-hydroxysuccinimide (0.05 mole) in AcOEt (150 ml) was added an EtOAc solution (50 ml) of N,N'-dicyclohexylcarbodiimide (DCC) (0.05 mole). The mixture was stirred at room temperature for 2 hr and allowed to stand overnight in a refrigerator. After AcOH (0.6 ml) was added, the mixture was stirred for 20 min. N,N'-Dicyclohexylurea was removed by filtration and washed with AcOEt. The combined filtrate and washings were washed successively with H_2O, 5% $NaHCO_3$ and H_2O, and dried over $MgSO_4$. The solvent was evaporated *in vacuo* to yield a crystalline residue. Recrystallization from AcOEt with petr. ether yielded product (VI). In a similar manner, VI' and VI" were prepared from V' and V", respectively (Table III).

DL-3-Hydroxyacyl-L-leucine Hydrazides (VIIIa—j)——a) DL-3-Hydroxyacyl-L-leucine Methyl Esters (VIIa—j): i) From IV: To a solution of IV (0.023 mole) dissolved in a mixture of glac. AcOH (70 ml) and conc. HCl (12 ml) was added aqueous 8.3 M $NaNO_2$ solution (3 ml) at −5——10° and the solution was stirred for 10 min, diluted with ice-water (100 ml) and extracted with cold AcOEt (250 ml). The AcOEt extract was washed successively with ice-cooled saturated NaCl solution, 1 N $NaHCO_3$ and H_2O, and dried over $MgSO_4$ in a cold room at 0°. This solution containing the azide was then combined with a solution of L-leucine methyl ester[21] (0.023 mole) in AcOEt (10 ml). The mixture was stirred at −5° for 1 hr and kept overnight in a refrigerator and for additional 2 days at room temperature. The reaction mixture was washed successively with 1 N HCl, 1 N $NaHCO_3$ and H_2O, dried over $MgSO_4$ and evaporated *in vacuo* to give oily product (VII) in 69—80% yields.

ii) From VI: A solution of VI (0.017 mole) and L-leucine methyl ester (0.017 mole) in AcOEt (30 ml) was stirred at room temperature for 3 hr and allowed to stand for additional 16 hr. The subsequent purification procedure was the same as described in i). Yields of the oily products, 83—99%.

b) DL-3-Hydroxyacyl-L-leucine Hydrazides (VIIIa—j): To a solution of VII (0.023 mole) in MeOH (60 ml) was added hydrazine hydrate (5.5 ml) and the reaction mixture was allowed to stand overnight at room temperature. The solvent was evaporated *in vacuo*, and H_2O (50 ml) was added to the residue. After cooling, the resulting crystal was collected by filtration, washed with H_2O and dried over P_2O_5 *in vacuo*. Yields were in a range of 57—88% (from IV or VI).

Resolution of VIIIa—j——VIII was dissolved in an appropriate alcohol shown in Table IV and the solution was kept overnight in a refrigerator. The crystals thus formed were collected by filtration and recrystallized three times from the same solvent used above to give L-3-hydroxyacyl-L-leucine hydrazide (IX) as colorless needles (Table IV). The mother liquor was concentrated to dryness under reduced pressure to give a solid residue which was dissolved in a small amount of the same alcohol as above, and then the solution was cooled slowly to 0°. The mass of opaque crystals which separated was filtered off and the filtrate was concentrated *in vacuo* to give a solid material. After the same procedure was repeated once again, the material was further reprecipitated twice from suitable solvent shown in Table V to yield D-3-hydroxyacyl-L-leucine hydrazide (X) as colorless powder (Table V).

D- or L-3-Hydroxyacyl-L-leucine Hydrazides (IX or X) from VI' or VI"——Using essentially the same procedure as described for the synthesis of VIII [method a)-ii)], the ester (VI' or VI") of the optically active acid was allowed to react with L-leucine methyl ester and the resulting product was hydrazinolyzed to give IX or X (Table IV and V).

Antibacterial Test (Table VI)——The minimum amount of a compound necessary for complete inhibiton of bacterial growth was determined by a standard two fold tube dilution method using a bouillon agar (in *E. coli*, or *St. aureus*), 1% glucose added-bouillon (in *Candida albicans*) and 10% albumin added-Dubos media (in *Mycobact. tuberculosis*).

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