N-Acyl-L-Leucines of Biologically Active Uncommon Fatty Acids: Synthesis and Antibacterial Activity

S.Y. Mhaskar[a], **G. Lakshminarayana**[a,*] and **L. Saisree**[b]

[a]Oils and Fats Division, Indian Institute of Chemical Technology, Hyderabad—500 007, India and [b]Centre of Cellular and Molecular Biology, Hyderabad—500 007, India

The effect of structural variation in the fatty acid chain on the antibacterial activity of *N*-acyl-L-leucines was investigated. Pure *N*-acyl leucines of some structurally different and biologically active uncommon fatty acids were synthesized for the first time and tested for antimicrobial activity. *N*-Stearoyl-, *N*-oleoyl- and *N*-ricinoleoyl leucines were also evaluated for comparison. The *N*-acyl leucines exhibited greater activity in acid form than the methyl ester form and against gram positive bacteria than gram-negative bacteria. The presence of a cyclopropane or a hydroxy group or unsaturation in the acyl chain increased the antibacterial activity. Shifting the hydroxyl group toward the amide linkage resulted in a diminished effect on the antibacterial activity.

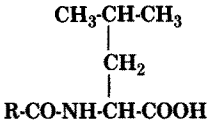
KEY WORDS: Biological activity, *N*-acyl leucines, structure *vs.* antibacterial activity, synthesis, uncommon fatty acids.

N-Acylamino acid derivatives exhibit both surfactant and antibacterial activities and are also skin-compatible (1). Hence, they find many applications in cosmetic and antibacterial formulations. Fieser *et al.* (2) synthesized *N*-acyl-amino acids for use as fat emulsifying agents. Kameda *et al.* (3) reported on the strong sterilization action of *N*-lauroyl-*DL*-phenylalanine. Fosdick *et al.* (4) found sodium *N*-acyl sarcosinate, a commercially used antibacterial agent, to possess strong anticaries action. Infante *et al.* (5) investigated the physicochemical and antimicrobial properties of *N*-acyl arginine esters of common fatty acids.

The synthesis of *N*-acyl-L-leucines of some uncommon fatty acids and correlation of the acyl structure with antibacterial activity form the subject of the present communication. We have selected L-leucine as the amino acid moiety because it showed the highest antibacterial activity of the diethanolamides of lauroyl dipeptides for five simple amino acids, namely glycine, leucine, phenylalanine, proline and cysteine (6). The uncommon fatty acids coupled with leucine were: 1,9-nonanedicarboxylic (azelaic), 9-octadecynoic (stearolic), 9,10-oxiraneoctadecanoic (9,10-epoxystearic), 9,10-methyleneoctadecanoic (dihydrosterculic), 5-*trans*-octadecen-7,9-diynoic (7), 6-hydroxy-7,9-octadecynoic (Mhaskar, S.Y., unpublished data) and its *R*-isomer (8) (Table 1). Azelaic acid was reported to show antimicrobial, cytotoxic and therapeutic effects in skin disorders (9). Dihydrosterculic acid, which contains a cyclopropane ring, was observed to inhibit the β-oxidation enzyme system (10). The acetylenic fatty acids are known to be more active against fungi than ethylenic fatty acids (11). The novel diacetylenic fatty acids, isolated from root bark of *Paramacrolobium caeruleum*, in-

TABLE 1

N-Acyl-L-Leucines



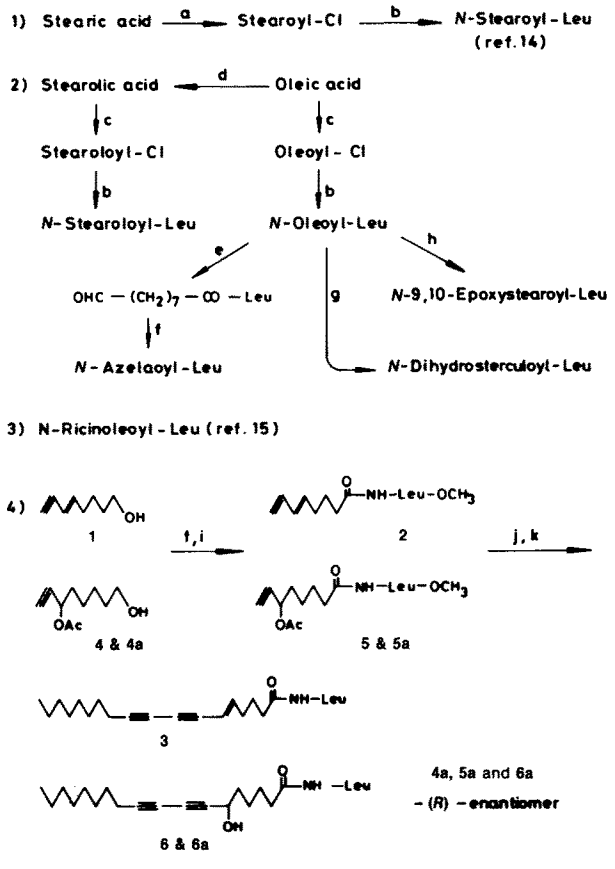
Acyl moiety	R-
Azelaoyl	$HOOC-(CH_2)_7-$
Stearoloyl	$CH_3-(CH_2)_7-C\equiv C-(CH_2)_7-$
9.10-Epoxystearoyl	$CH_3-(CH_2)_7-CH(O)CH-(CH_2)_7-$
Dihydrosterculoyl	$CH_3-(CH_2)_7-CH(CH_2)CH-(CH_2)_7-$
5-*trans*-Octadecen-7,9-diynoyl	$CH_3-(CH_2)_7-C\equiv C-C\equiv C-CH=CH-(CH_2)_3-$
6-Hydroxy-7,9-octadecadiynoyl	$CH_3-(CH_2)_7-C\equiv C-C\equiv C-CH(OH)-(CH_2)_4-$
6-(*R*)-Hydroxy-7,9-ocatdecadiynoyl	$CH_3-(CH_2)_7-C\equiv C-C\equiv C-CH(OH)-(CH_2)_4-$
Stearoyl	$CH_3-(CH_2)_{16}-$
Oleoyl	$CH_3-(CH_2)_7-CH=CH-(CH_2)_7-$
Ricinoleoyl	$CH_3-(CH_2)_4-CH(OH)-CH_2-CH=CH-(CH_2)_7-$

*To whom correspondence should be addressed.

hibit the activity of HMG-CoA reductase, the enzyme involved in cholesterol biosynthesis (12). The leucine derivatives of common fatty acids, namely stearic (octadecanoic), oleic (9-*cis*-octadecenoic) and ricinoleic (12-hydroxy-9-*cis*-octadecenoic) acids were also synthesized and compared in primary screening for antibacterial activity.

MATERIALS AND METHODS

Stearic and oleic acids were purchased from BDH Ltd. (Poole, England). The synthetic routes followed for the compounds investigated are shown in Scheme 1,



SCHEME 1

where the conditions were: a) $SOCl_2$, 4h; b) i. aq. NaOH solution, L-leu; ii. dil. HCl; c) $C_2O_2Cl_2$, C_6H_6, 4h; d) i. Br_2, CCl_4, 0°C; ii. KOH, *n*-propanol, dimethyl sulfoxide; e) OsO_4, $NaIO_4$, THF/Water (4:1); f) Jones' reagent, acetone, 0°C; g) CH_2N_2, $Pd(OAc)_2$, ether, 0°C; h) MCPBA, dry DCM, 0°C; i) C_2H_5OCOCl, $(C_2H_5)_3N$, dry THF, L-leu-OMe, 0°C; j) 1-bromo-1-decyne, $(Ph_3P)_2PdCl_2$, $(C_2H_5)_2NH$, CuI, C_6H_6; k) i. aq. NaOH solution; ii. dil. HCl. Stearolic acid was prepared from oleic acid as reported in the literature (13). For the synthesis of *N*-[5-*trans*-octadecen-7,9-diynoyl] leucine (**3**), the fragment 5-*trans*-octen-7-ynol (**1**) was synthesized as described by us (7) for use as starting material. For *N*-[6-hydroxy-7,9-octadecadiynoyl] leucine (**6**) and *N*-[6-(R)-hydroxy-7,9-octadecadiynoyl] leucine (**6a**), the fragments 6-hydroxy-7-octynol (**4**) and its *R*-enantiomer (**4a**) were synthesized, respectively, for use as starting materials (8). Analytical-grade reagents required for the synthesis were purchased from Aldrich Chemical Co. (Milwaukee, WI). Chemicals and solvents of analytical grade were purchased from Indian Drug and Pharmaceutical Ltd. (Hyderabad, India). Tetrahydrofuran (THF) was distilled and dried over sodium metal. Silica gel (60–120 mesh) for column chromatography was obtained from ACME Synthetic Chemicals (Bombay, India).

Gas-liquid chromatography (GLC) was carried out on a Silar 10 C column in a Hewlett-Packard 5840 A (Hewlett-Packard Co., Palo Alto, CA) chromatograph fitted with a hydrogen flame detector and a data processor. The column, injection port and detector were maintained at 195, 250 and 300°C, respectively. Flow rate of carrier gas (N_2) was 30 mL. Infrared (IR) spectra were recorded on a Perkin-Elmer 683 spectrophotometer (Perkin-Elmer, Norwalk, CT). Proton nuclear magnetic resonance (NMR) spectra were obtained in $CDCl_3$ on a Varian 60-FT (Varian Associates, Palo Alto, CA).

Synthesis of N-*stearoyl-, N-oleoyl- and* N-*stearoloyl leucine.* The acid chloride of stearic acid was prepared with thionyl chloride. For unsaturated fatty acids, oxalyl chloride was used. The acid chlorides were distilled under vacuum. *N*-Acyl leucines were prepared by the Schotten-Baumann reaction by adding acid chloride dropwise to an aqueous solution of the sodium salt of leucine at pH 10 ± 0.5 (14). The product was acidified with 30% H_2SO_4, taken up in ethyl acetate and crystallized by addition of hexane. *N*-Oleoyl leucine was purified on a silica gel column with *n*-hexane/ethyl acetate (90:10, vol/vol). *N*-Stearoyl, *N*-oleoyl and *N*-stearoloyl leucines were obtained in 70–80% yield.

Synthesis of N-*ricinoleoyl leucine (15).* Ricinoleic methyl ester was isolated from methyl esters of castor oil fatty acids by silica gel column chromatography. The hydroxyl group was protected by formation of the tetrahydropyranyl ether (O-THP), and then the methyl ester was hydrolyzed to form the acid. The O-THP-protected acid was converted into the anhydride by use of dicyclohexylcarbodiimide (DCC). The anhydride was treated with leucine methyl ester (H_2N-leu-OMe) in the presence of 4-dimethylaminopyridine (DMAP) to yield methyl ester of *N*-ricinoleoyl leucine, which upon saponification followed by acidification yielded the title product.

Synthesis of N-*azelaoyl leucine. N*-Oleoyl leucine (10 mmol) was taken up in a THF-water mixture (4:1, 20 mL), and crystals of osmium tetroxide were added. After separation of a brown mass in 5 min, $NaIO_4$ (20 mmol) was added. After 1 h of stirring at room temperature, THF was evaporated and the product was extracted with ethyl acetate. *N*-[9-Oxo-nonanoyl] leucine was purified on a silica gel column, taken up in acetone and then cooled to 0°C. Jones reagent was added dropwise until the reaction mixture retained the orange color of the reagent for 30 min. The excess reagent was quenched with isopropanol. The title product was separated on a silica gel column in 95% yield.

Synthesis of N-*9,10-epoxystearoyl leucine. N*-Oleoyl leucine (10 mmol) was dissolved in dry DCM (20 mL), and *m*-chloroperbenzoic acid (MCPBA) (11 mmol) was added. After stirring for 3 h, dichloromethane (DCM) was evaporated. The crude product along with solid *m*-chlorobenzoic acid was loaded and separated on a silica gel column to yield 90% of the title product.

Synthesis of N-*dihydrosterculoyl leucine. N*-Oleoyl leucine (1 mmol) and a catalytic amount of palladium (II)

acetate in diethyl ether (15–20 mL) was cooled to 0°C, and a methanolic ether solution of diazomethane (20–25 mL) was added dropwise with continuous stirring during 10 min. After removal of the solvent, the product was purified on a silica gel column to give 70% of the title product.

Synthesis of N-[*5*-trans-*octadecen-7,9-diynoyl*]-, N-[*6-hydroxy-7,9-octadeca-diynoyl*]- *and* N-[*6-(R)-hydroxy-7,9-octadecadiynoyl*] *leucines (3, 6 and 6a).* Fragments **1, 4** and **4a** were synthesized as per the procedure already reported by us (7,8); **1, 4** or **4a** (5 mmol) was dissolved in acetone, and Jones reagent was added at 0°C until the reagent color was retained for 30 min. Excess reagent was quenched with isopropanol. The product was extracted with ether, washed with water and isolated to give the acid in 95% yield. The acid (4 mmol), triethylamine (6 mmol) and ethyl chloroformate (6 mmol) were stirred in dry THF for 30 min at −5°C. The leucine methyl ester (5 mmol) was added under N_2 atmosphere. The stirring was continued for another 3 h, after which THF was evaporated. The product was extracted with ether and washed with water. Solvent was evaporated, and the product was purified on a silica gel column. From the fragments **1, 4** and **4a**, the products **2, 5** and **5a** were obtained, respectively, in 90–95% yield. These compounds were coupled with 1-decyne for formation of the title products. 1-Bromodec-1-yne (2.5 mmol) was stirred in benzene (5 mL) with continuous N_2 bubbling through the solution to deaerate it, and then *bis*(triphenylphosphine)palladium dichloride (0.15 mmol) was added (16). After 15 min, CuI (0.4 mmol) and diethylamine (3 mmol) were added and stirred for 15 min. The solution of **2, 5** or **5a** (2.5 mmol) in benzene (2 mL) was added dropwise, and the reaction mixture was stirred for 5 h. The reaction was quenched with an aqueous NH_4Cl solution. The benzene layer was washed with water, dried over Na_2SO_4 and evaporated, and the crude product was purified on a silica gel column to give methyl esters of **3, 6** or **6a** in *ca.* 33% yield. These esters, upon base hydrolysis followed by acidification as described earlier, offered the title products, **3, 6** and **6a** in 95% yield.

Antibacterial properties. Cultures of *Staphylococcus aureus* and *Escherichia coli* were grown overnight to a stationary phase at 37°C in a nutrient broth (composition: peptone 0.5%, beef extract 0.3% and NaCl 0.5%). Portions (0.1 mL) of the grown cultures were spread on plates of nutrient agar (composition: the same as for the broth with additional 1.5% agar). After drying the plates in a sterile hood for 15 to 30 min, sterile filter paper discs (5 mm diameter) were placed with sterile forceps. A solution of the sample (10 μL of 1% methanol solution equivalent to 100 μg/disc) was spotted on the paper disc. The plates were placed in the incubator overnight at 37°C. The diameter of the zone of inhibition was obtained from the average of three determinations.

Minimum inhibitory concentration (MIC). The culture was grown overnight, and the next day this culture was diluted 20 times with nutrient broth. This mixture was incubated for 1 to 2 h to get the mid log-phase culture (the phase in which the multiplication of bacteria is maximum). This mid log-phase culture was diluted 25 times with nutrient broth medium, and 0.2 mL was added to each well (0.3 mL capacity), except for the first well in a microtitre plate. In the first well, 0.3 mL culture and 15 μL of the sample solution were placed. This was thoroughly mixed, and 0.1 mL of the mixture was transferred to the next well. Likewise, wells with further serial dilution (*i.e.,* 0.1 mL from the second well to the third well and so on) were incubated overnight at 37°C. Next day, the wells were viewed for their clarity. The clarity of the solution shows the inhibition of bacterial growth at a particular concentration of sample. The last well showing clarity in the series gives the MIC for the particular sample.

RESULTS AND DISCUSSION

Synthesis of N-*acyl leucines.* Purity of all *N*-acyl leucines was confirmed by single spots from thin-layer chromatography (TLC). Also, NMR and mass spectra (MS) analyses showed that these compounds did not contain either free amino acid or fatty acid. Using azelaic acid for acylation was impossible due to lack of differentiation between the two carboxyls, only one of which was to be acylated. Hence, synthesis started with *N*-oleoyl leucine, which on cleavage of the double bond with $OsO_4/NaIO_4$ and Jones oxidation gave *N*-azelaoyl leucine. Acylation of leucine with 9,10-epoxystearic acid was not achieved due to opening of the oxirane ring. Hence, *N*-oleoyl leucine was epoxidized by MCPBA to give N-9,10-epoxystearoyl leucine in 90% yield. The same strategy, *i.e.,* constructing the necessary structure in the end, was applied in the synthesis of *N*-dihydrosterculoyl leucine by cyclopropanation with $CH_2N_2/Pd(OAc)_2$. In the synthesis of **3, 6** and **6a**, the fragment C_1-C_8 was first coupled with leucine methyl ester so as to keep the low-yielding acetylenic coupling of the C_1-C_8 fragment with 1-decyne at the end of the synthesis. Fragments **1, 4** and **4a** were oxidized by Jones reagent to respective acids, which were coupled with leucine methyl ester by the carboxylic-carbonic anhydride method in quantitative yield. The (C_1-C_8)-leu-OMe fragments (**2, 5** and **5a**) were extended with the 1-decyne chain by means of Sonogashira's method of palladium-catalyzed acetylenic coupling. This coupling gave 30% yield. The maintenance of an inert atmosphere was essential because the presence of O_2 reduced the yield. The final products **3, 6** and **6a** were obtained by base hydrolysis of the ester followed by acidification. This step also removed the acetate protection of the hydroxyl group (at 6-C in the acyl chain) in **5** and **5a**.

Spectral properties. IR, NMR and mass spectra of all the compounds showed many common peaks, along with some additional peaks pertaining to uncommon functional groups. The IR spectra showed absorptions at 2920 cm^{-1} (alkyl stretching), 1460 cm^{-1} (alkyl bending), 1670 cm^{-1} (secondary amide stretching) and 1720 cm^{-1} (acid carbonyl stretching). The epoxy and cyclopropane groups were seen at 830 cm^{-1} and 1440 cm^{-1}, respectively. The hydroxyl group was seen as a broad band at 3500 cm^{-1}. The conjugated diacetylenic functionality was observed as a sharp peak at 2660 cm^{-1}.

^{1}H-NMR spectra (δ ppm) of all the condensates showed common peaks in *N*-acyl leucines as follows: δ 5.5–7 (*d*, 1H, -NH-), 4.51 (*m*, 1H, -C*α*H), 2.2 (*s*, 2H, $CO\text{-}CH_2$-), 1.25 (*s* merged with *m*, n × 2H, $[\text{-}CH_2\text{-}]_n$), 0.96 (*d*, 6H, $(CH_3)_2$-CH), 0.88 (*t*, 3H, CH_3-CH_2). The NMR data related to different functionalities in acyl moieties are given in Table 2.

Antibacterial activity. The antibacterial activities of *N*-

TABLE 2

^{1}H-Nuclear Magnetic Resonance Data (δ ppm) for *N*-Acyl-L-Leucines

Acyl moiety	Characteristic peaks
Azelaoyl	2.2 (*t*, 2H, -CH_2-COOH)
Stearoloyl	2.2 (*t*, 4H, -CH_2-C≡)
9.10-Epoxystearoyl	3.0 (*bm*, 2H, -CH-CH- of epoxy ring)
9,10-Dihydrosterculoyl	0.65 (*m*, 2H, -CH-CH- of cyclopropane ring), 0.56 & −0.33 (*m*, 2H, CH_2 protons of cyclopropane ring)
5-*trans*-Octadecen-7,9-diynoyl	6.3 (double *t*, 1H, -CH=), 5.5 (*bd*, 1H, =CH-) 2.3-2.1 (*m*, 4H, CH_2-C≡, CH_2-CH=)
6-Hydroxy-7,9-octadecadiynoyl or its *R*-enantiomer	4.4 (*bt*, 1H, ≡C-CH-O), 2.2 (*m*, 2H, ≡C-CH_2)
Oleoyl	5.4 (*m*, 2H, -CH=CH-), 2.1 (*m*, 4H, -CH_2-CH=)

TABLE 3

Antibacterial Activity[a] of *N*-Acyl-L-Leucines[b] by the Disc Method

Acyl moiety	*Staphylococcus aureus* diameter (cm)	*Escherichia coli* diameter (cm)
Azelaoyl	1.5	1.3
Stearoloyl	2.4	1.4
9,10-Epoxystearoyl	1.4	—
Dihydrosterculoyl	2.6	1.7
5-*trans*-Octadecen-7,9-diynoyl	2.2	1.0
6-Hydroxy-7,9-octadecadiynoyl	1.2	—
6-(*R*)-Hydroxy-7,9-octadecadiynoyl	1.1	—
Stearoyl	—	—
Oleoyl	—[c]	—
Ricinoleoyl	2.0	—
Streptomycin (control)	2.2	2.1

[a]Activity was measured as diameter of the inhibited zone with methanol as control.
[b]*N*-Acyl leucines were spotted as 10 mg/mL in methanol.
[c]Activity was seen above 20 mg/mL concentration.

TABLE 4

Minimum Inhibitory Concentration (MIC)[a] of *N*-Acyl-L-Leucines

Acyl moiety	*Staphylococcus aureus* (mg/mL)	*Escherichia coli* (mg/mL)
Azelaoyl	0.75	1.4
Stearoloyl	0.06	1.0
9,10-Epoxystearoyl	0.75	—
Dihydrosterculoyl	0.04	1.0
5-*trans*-Octadecen-7,9-diynoyl	0.27	5.0
6-Hydroxy-7,9-octadecadiynoyl	7.0	10.0
6-(*R*)-Hydroxy-7,9-octadecadiynoyl	7.0	10.0
Stearoyl	—	—
Oleoyl	12.0	20.0
Ricinoleoyl	1.0	—

[a]MIC was determined based on retention of clarity of the nutrient medium during inhibition of bacterial growth.

acyl leucines against *Staphylococcus aureus* (gram-positive) and *Escherichia coli* (gram-negative) are tabulated in Tables 3 and 4. The screening of activity was done on the solutions in methanol. The antibacterial activity of these derivatives was more pronounced in acid form than in the methyl ester form of leucine. In general, the activity of *N*-acyl leucines was greater against the gram-positive than gram-negative bacteria (Table 3). The gram-negative bacteria were generally resistant or were little inhibited by these derivatives. As the unsaturation in the acyl chain changed, from saturated (stearoyl) to olefinic (oleoyl) to acetylenic (stearoloyl), the antibacterial activity of *N*-acyl leucines increased. The presence of additional unsaturation, as in 5-*trans*-octadecen-7,9-diynoic acid moiety (two acetylene bonds and one *trans* olefinic bond) did not increase this effect compared to stearolic moiety (2.2 *vs.* 2.4 cm). The presence of an additional carboxylic group as in azelaic acid (1.5 cm) enhanced the inhibition compared to stearic, which showed no activity. However, the effect was still less than that of the acetylenic bond (2.4 cm). Conversion of a double bond into an epoxy ring, as in 9,10-epoxystearic acid (1.4 cm), showed higher inhibitory activity of the oxirane ring. The maximum inhibition of *N*-acyl leucine of cyclopropane fatty acid (2.6 cm), compared to other acyl leucines, highlighted the effect of this cyclic system. Use of ricinoleic acid as acyl moiety confirmed the favorable effect of the hydroxyl group on inhibition (2.0 cm) when compared to oleic acid. The effect was almost the same as that of the two acetylenic bonds in 5-*trans*-octadecen-7,9-diynoyl group (2.2 cm). The change in position of the hydroxyl group from the twelfth to the sixth carbon in the acyl chain reduced the antibacterial activity of *N*-(6-hydroxy-7,9-octadecadiynoyl) leucine (1.2 cm) in spite of the presence of two acetylenic bonds. This also brought out the importance of the double bond for antibacterial activity. The (*R*)-enantiomer of the 6-hydroxy-7,9-octadecadiynoic moiety did not produce any change in antibacterial activity, showing that inhibition of this acid is not enantiospecific. Streptomycin, the control, had inhibition zones of 2.2 and 2.1 cm for gram-positive and gram-negative bacteria, respectively.

The MICs of the *N*-acyl leucines are given in Table 4. In general, the MICs of these derivatives in acid form were much higher for gram-negative than for gram-positive bacteria as expected from their zone of inhibition data. Increased unsaturation from stearoyl to stearoloyl group decreased the MICs and exhibited a minimum for the acetylenic bond (0.06 mg/mL). The presence of terminal free carboxylic group in *N*-azelayol leucine and the epoxy ring in *N*-9,10-epoxystearoyl leucine showed MIC values at 0.75 mg/mL. This was slightly higher than for stearoloyl leucine (0.06 mg/mL) but still less compared to oleoyl (12.0 mg/mL). The dihydrosterculoyl moiety, as expected from the zone of inhibition, exhibited the lowest MIC (0.04 mg/mL) among all the acyl moieties, highlighting the potent antibacterial nature of the cyclopropane ring. Increased unsaturation in the 5-*trans*-octadecen-7,9-diynoyl acyl moiety (0.27 mg/mL) increased MIC slightly more than the stearoloyl moiety (0.06 mg/mL). Presence of the hydroxyl group at the twelfth carbon in the ricinoleoyl moiety, in addition to the double bond as in the oleic moiety, lowered the MIC from 12.0 mg/mL to 1.0 mg/mL. The effect of a shift in the position of the hydroxyl group to the sixth carbon atom as in 6-hydroxy-7,9-octadecadiynoic acid, which is nearer to the amide bond of the derivative suppressed even the effect of the presence of its two acetylenic bonds on MIC (7.0 mg/mL

vs. 1.0 mg/mL for ricinoleoyl derivative). The enantiomer showed the same activity as the racemic mixture, indicating that the MIC is not dependent on the enantiomeric configuration of 6-hydroxy-7,9-octadecadiynoyl moiety.

In conclusion, the presence of unsaturation, cyclopropane or hydroxyl groups in the acyl moiety of *N*-acyl leucines increased the antibacterial activity and lowered MIC. A shift in the position of the hydroxyl group toward the amide linkage decreased the antibacterial activity. In general, these derivatives are active in their acid form against gram-positive bacteria.

ACKNOWLEDGMENT

This is communication # 2986 from the Indian Institute of Chemical Technology (CSIR).

REFERENCES

1. Linfield, W.M., *Anionic Surfactants; Vol. 7, Part II,* Surfactant Science Series, Marcel Dekker, Inc., New York, 1976, pp. 582–612.
2. Fieser, M., L.F. Fieser, E. Tbromanoff, Y. Hirata, H. Heymann, H. Teff and S. Bhattacharya, *J. Am. Chem. Soc. 78:*2825 (1956).
3. Kameda, Y., E. Toyoura, S. Ohshima, M. Tsuji and C. Irie, *J. Pharm. Soc. Japan 68:*143 (1948).
4. Fosdick, L.S., J.C. Calandra, R.O. Blackwell and J.H. Burill, *J. Dental Res. 32:*486 (1953).
5. Infante, R., J.G. Dominguez, P. Erra, R. Julia and M. Prats, *Int. J. Cosmet. Sci. 6:*275 (1984).
6. Mhaskar, S.Y., and G. Lakshminarayana, *J. Am. Oil Chem. Soc. 69:*643 (1992).
7. Mhaskar, S.Y., and G. Lakshminarayana, *Synth. Commun. 20:*2001 (1990).
8. Mhaskar, S.Y., and G. Lakshminarayana, *Tetrahedron Lett. 31:*7227 (1990).
9. Nazzaro-Porro, M., *J. Am. Acad. Dermatol. 17:*1033 (1987).
10. Wood, R., and R. Rieser, *J. Am. Oil Chem. Soc. 42:*315 (1965).
11. Kabara, J.J., in *The Pharmacological Effects of Lipids,* edited by J.J. Kabara, American Oil Chemists' Society, Champaign, 1978, pp. 1–14.
12. Patil, A.D., J.A. Chan, P. Lois-Flamberg, R.J. Mayer and J.W. Westley, *J. Nat. Prod. 52:*153 (1989).
13. Silbert, L.S., *J. Am. Oil Chem. Soc. 61:*1090 (1984).
14. Jungermann, E., J.F. Gerecht and I.J. Krems, *J. Am. Chem. Soc. 78:*172 (1956).
15. Mhaskar, S.Y., and G. Lakshminarayana, *Synth. Commun. 20:*1305 (1990).
16. Sonogashira, K., Y. Tohda and N. Hagihara, *Tetrahedron Lett. 50:*4467 (1975).

[Received March 9, 1992; accepted October 1, 1992]