

## NMR and HPLC characterisation of *O*-alkanoyl lactates prepared by lipase catalysis

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Lactic acid esters of organic acids like lauroyl, palmitoyl and stearyl lactic acids prepared by using lipases have been characterized by  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra. The reaction mixture indicates the presence of several species due to complex esterification equilibria. Crystalline lactic acid employed for reactions shows more lactylate content than the commercially available lactic acid. The  $-\text{CH}-\text{O}-$  region between 4.03 and 5.13 ppm gives quite a lot of information on the ester formation as this region contains signals from free lactic acid, hydroxyl terminal group of lactylates, carboxyl terminal group of lactylates and lactic acid and lactylate esters of organic acids employed. The ester yields determined by HPLC and NMR are comparable and are less than those determined by titration method.

Fatty acid (*O*-alkanoyl) esters of hydroxy acids like lactic acid are surface active compounds used extensively in the manufacture of bakery products like cakes and doughnuts, dairy products like ice cream, and also as bactericides and fungicides<sup>1,2</sup>. A few chemical methods are available for the preparation of *O*-alkanoyl lactates<sup>3,4</sup>. An alternative enzymatic method involving lipase was investigated by us for the preparation of *O*-alkanoyl lactates<sup>5,6</sup>.

The reaction is quite complex due to inter-esterification reactions of lactic acid itself leading to lactylates and further reactions of free hydroxyl groups of lactic acid and lactic acids with carboxyl groups of organic acids. The extent of product formation determined by titration method was always found to be higher than that determined by  $^1\text{H}$  NMR and HPLC which indicated that the above esterification gives rise to a mixture of esterified products. The products formed required a thorough investigation and hence,  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectroscopic studies were undertaken.

### Results and Discussion

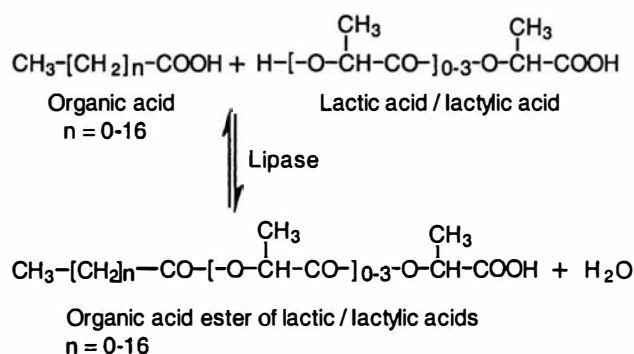
Lactic acid esters of organic acids were prepared under various conditions using lipases **Scheme I**. Formation of ester during the course of the reaction was monitored by a back titration procedure, which measured only decrease in the total acid content. When reaction was conducted with lactic acid alone, the decrease in acid content upto an extent of 22.9 %

was observed after 72 hr indicating formation of lactic acid oligomers. However, the titration method did not give indication of extent of esterification unequivocally. Hence, detailed  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopic studies along with HPLC were employed to characterize the products formed and the results are discussed in detail below.

### HPLC

Calibration plots were constructed for lactic acid and other organic acids like stearic, palmitic, lauric, decanoic, octanoic, valeric, isovaleric, butyric, isobutyric, propionic and acetic acids, in the concentration range 0-25  $\mu\text{g}/\mu\text{L}$  from which concentrations of unreacted acids present in the reaction mixture were determined.

HPLC chromatogram of lactic acid showed two peaks, one corresponding to free lactic acid at 4.8 min



**Scheme I**— Lipase catalyzed esterification of organic acids with lactic/lactic acids.

and the other to higher oligomers of lactic acid (lactylates) at 3.3 min. Retention time was found to increase with the increase in carbon chain length of the acids. In the case of stearic acid, two peaks were observed at 13.5 and 21.8 min corresponding to palmitic and stearic acids, respectively, showing that stearic acid contained small amount of palmitic acid. The difference between the total amount of the sample from the reaction mixture injected and the concentrations of unreacted lactic acid and fatty acid gave the actual concentration of the ester. Ester yields and concentrations of unreacted acids are given in **Table I**. Organic acid esters of lactic acid were found to elute at the same retention time as lactic acid esters, at 3.3 min. Hence, increase in peak area at 3.3 min indicated the formation of both lactic and lactylic acid esters of organic acids. **Figures 1a** and **1b** show HPLC chromatograms of stearyl lactic acid and commercial sodium stearyl lactylate.

HPLC analysis clearly indicated that the reaction mixture contained lactic, lactylic and organic acids and organic acid esters of lactic and lactylic acids.

#### <sup>1</sup>H NMR of lactic acid

Oligomers upto a pentamer have been reported in commercial lactic acid<sup>7</sup> **Scheme II**. Hence a detailed NMR investigation of lactic acid itself was carried out. <sup>1</sup>H NMR spectrum of commercial lactic acid recorded in DMSO-*d*<sub>6</sub> is shown in **Table II**. There are two major doublets at 1.22 and 1.38 ppm, corresponding to -CH<sub>3</sub> of free lactic acid **1** and -CH<sub>3</sub> of the carboxyl terminal lactic acid group of the lactylates **3**, respectively. Another doublet at 1.26 ppm corre-

sponded to the -CH<sub>3</sub> of hydroxyl terminal lactic acid group of the lactylates **2**. Along with these, there are other minor -CH<sub>3</sub> doublets (1.41-1.43 ppm) observed, corresponding to mixture of various other lactylates with two or more lactic acid molecules condensed together as lactylic acid esters (**4,5,n**). There are two sets of quartets for the -CH-O- signal at 4.05 ppm and 4.18 ppm corresponding to free lactic acid **1** and that from free hydroxyl terminal lactic acid group of the lactylates **2**. The quartets at 4.90 ppm and beyond correspond to the -CH-O- of the lactate esters of the lactylates **3** **Table II**. The proportions of lactylate -CH-O- (4.9 - 5.08 ppm and beyond) and free lactic acid -CH-O- (4.05 ppm) determined from the respective areas of these signals were found to be 22.7% and 77.3%, respectively. Thus, commercial lactic acid in which 12 % water is present, contains 68.1 % free lactic acid and 19.9 % lactylates, which corresponded well with those determined by potentiometric method (61.3 % of free lactic acid and 26.7 % of lactylates out of 88 %).

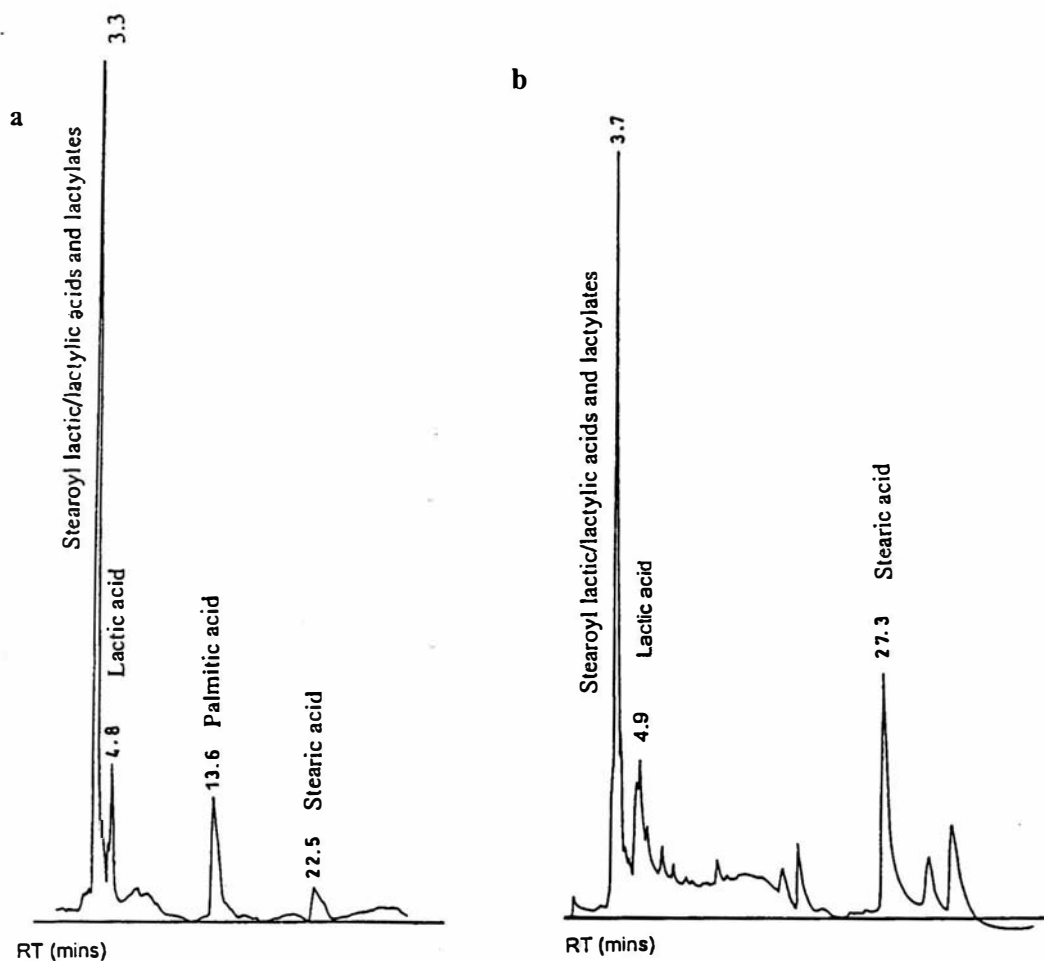
In the <sup>1</sup>H NMR spectrum of the crystalline lactic acid prepared from commercial lactic acid by azeotropic reflux with benzene to remove water, the area of the signal at 1.38 ppm was greater than that observed for the commercial lactic acid sample, indicating the presence of higher amounts of lactylic acid esters in crystalline lactic acid. Larger areas of two quartets present at 4.9 and 5.08 ppm due to -CH-O- of the esters also indicated the presence of higher amounts of lactylates **Table II**.

Here also, the proportions of free lactic acid and lactylates in crystalline lactic acid worked out from

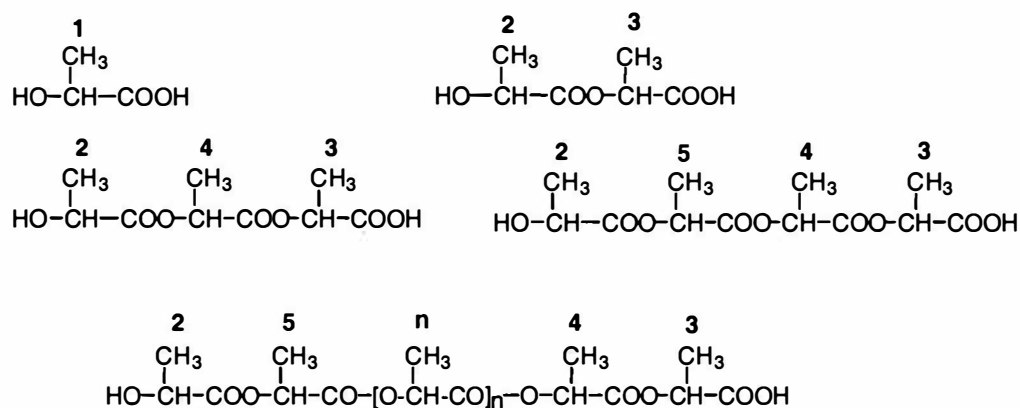
**Table I**—Characterization data of Fmoc-amino acid azides

| Sl No. | Compd                     | Yield<br>(%) | m.p.<br>°C | R <sub>f</sub> Value* |                  | [α] <sub>D</sub> <sup>25</sup><br>(c=1, CHCl <sub>3</sub> ) | Calc.%(Found)    |              |                |
|--------|---------------------------|--------------|------------|-----------------------|------------------|-------------------------------------------------------------|------------------|--------------|----------------|
|        |                           |              |            | R <sub>f</sub> A      | R <sub>f</sub> B |                                                             | C                | H            | N              |
| 1      | Fmoc-Gly-N <sub>3</sub>   | 90           | 132        | 0.61                  | 0.69             | -                                                           | 63.34<br>(63.01) | 4.37<br>4.19 | 17.38<br>17.03 |
| 2      | Fmoc-Ala-N <sub>3</sub>   | 93           | 162        | 0.62                  | 0.68             | -16                                                         | 64.27<br>(63.99) | 4.78<br>4.61 | 16.66<br>16.52 |
| 3      | Fmoc-Leu-N <sub>3</sub>   | 92           | 123        | 0.76                  | 0.68             | +7                                                          | 66.65<br>(66.20) | 5.85<br>5.70 | 14.80<br>14.61 |
| 4      | Fmoc-Phe-N <sub>3</sub>   | 94           | 175        | 0.61                  | 0.75             | +3                                                          | 69.88<br>(69.50) | 4.88<br>4.68 | 13.58<br>13.39 |
| 5      | Fmoc-L-Phg-N <sub>3</sub> | 90           | 125-26     | 0.63                  | 0.76             | -11                                                         | 69.33<br>(69.01) | 4.54<br>4.32 | 14.06<br>13.90 |
| 6      | Fmoc-D-Phg-N <sub>3</sub> | 89           | 127        | 0.63                  | 0.76             | +11                                                         | 69.33<br>(69.08) | 4.54<br>4.41 | 14.06<br>13.95 |

\*TLC analysis carried out using ethyl acetate-*n*-hexane (35:65, v/v) and CHCl<sub>3</sub>-methanol-acetic acid (40:2:1, v/v/v) and the R<sub>f</sub> values are designated as R<sub>f</sub> A & R<sub>f</sub> B respectively.



**Figure 1**—HPLC chromatograms of **a**—enzymatically prepared stearoyl lactic acid; **b**—commercial sodium stearoyl lactate (acidified). Conditions- column: C<sub>18</sub>-Shimpack-ODS; mobile phase: acetonitrile-orthophosphoric acid, 5 mM (90:10); detector: UV 205 nm. Stock solutions containing 10 µg/µl samples were prepared from which 10 µl samples were injected.



**Scheme II**—Different species of lactylates present in commercial and crystalline lactic acids. The number in bold indicate labelling **1**—free lactic acid; **2**—hydroxy terminal lactic acid group of lactylate oligomers; **3**—carboxyl terminal lactic acid group of lactylate oligomers; **4, 5, n**—ester lactic acid group of lactylates.

Table II—<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of commercial and crystalline lactic acid.

| Sample                  | <sup>1</sup> H NMR (δ, in ppm) |        |             |       |
|-------------------------|--------------------------------|--------|-------------|-------|
|                         | -CH <sub>3</sub>               |        | -CH-O-      |       |
| 88 % pure lactic acid   | 1.22 (5.8)                     | 1      | 4.05 (6.5)  | 1     |
|                         | 1.26                           | 2      | 4.18        | 2     |
|                         | 1.38                           | 3      | 4.90        | 3     |
|                         | 1.41-1.43                      | 4,5,n  | 4.96-5.08   | 4,5,n |
| Crystalline lactic acid | 1.21 (6.74)                    | 1      | 4.03 (6.75) | 1     |
|                         | 1.26                           | 2      | 4.16        | 2     |
|                         | 1.38                           | 3      | 4.90        | 3     |
|                         | 1.39-1.43                      | 4,5, n | 4.96-5.14   | 4,5,n |

| Sample                  | <sup>13</sup> C NMR (δ, ppm) |            |           |            |                   |        |       |   |
|-------------------------|------------------------------|------------|-----------|------------|-------------------|--------|-------|---|
|                         | -CH <sub>3</sub>             |            | -CH-O-    |            | -CO- <sup>a</sup> |        | -COOH |   |
| 88 % pure lactic acid   | 20.1                         | 1          | 66.9      | 1          | 175.2             | 2      | 178.1 | 1 |
|                         | 16.8                         | 2,3,4,5,n  | 69.7-70.1 | 2,3,4,5, n | 173.8             | 3      |       |   |
|                         |                              |            |           |            | 171.1             | 4,5,n  |       |   |
| Crystalline Lactic acid | 20.6                         | 1          | 66.9      | 1          | 175.0             | 2      | 178.3 | 1 |
|                         | 16.7-19.9                    | 2,3,4,5, n | 67.0-69.4 | 2,3,4,5,n  | 173.8             | 3      |       |   |
|                         |                              |            |           |            | 172.9-170.3       | 4,5, n |       |   |

1-free lactic acid; 2 - hydroxyl terminal lactic acid group of lactylate oligomers; 3 - carboxyl terminal lactic acid group of lactylate oligomers; 4,5,n-lactylates; 2,3,4,5..n: applicable only to lactylates; a- assignments are interchangeable

the areas of signals at 4.05 and 4.90-5.14 ppm were 48.9 % and 46.3 %, respectively along with 4.8 % water which corresponded with values determined by potentiometric measurements (43.7 % and 51.5 %, respectively).

### <sup>13</sup>C NMR of lactic acid

<sup>13</sup>C NMR spectrum of commercial lactic acid, showed two signals at 20.1 and 16.8 ppm, corresponding to -CH<sub>3</sub> of free lactic acid and that of the lactylates Table II respectively. In the -CH-O- region, there are three signals at 66.9, 69.7 and 70.1 ppm, the 66.9 ppm signal being the most prominent one, corresponding to -CH-O- of free lactic acid and the other two corresponding to lactylates Table II. In the carbonyl carbon region, the signal at 178.1 ppm corresponds to free lactic acid and the others at 175.2, 173.8 and 171.1 ppm correspond to lactylates Table II. The most upfield one is probably from the higher oligomers.

In case of crystalline lactic acid, the signal intensity corresponding to -CH<sub>3</sub> of free lactic acid (20.6 ppm) was less than that from the oligomeric esters (19.9 and 16.7 ppm), indicating higher proportions of oligomers than that present in the commercial sample. The carbonyl carbon region Table II signals designated as 2 and 3 also confirms this.

### <sup>1</sup>H NMR of organic acid esters of lactic acid

The organic acid esters of lactic acid were also characterized by <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy. <sup>1</sup>H NMR spectral data of reaction mixtures of lauroyl, palmitoyl and stearoyl lactic acids were recorded. A typical <sup>1</sup>H NMR spectral data of stearoyl lactic acid is shown in Table III. In all the cases, a triplet at 0.85 ppm corresponded to -CH<sub>3</sub> from free and esters of the fatty acid employed Table III. A huge signal envelope at 1.24 ppm corresponded to -CH<sub>2</sub>- groups of free and esterified fatty acid. The signal at 1.42 ppm corresponded to -CH<sub>3</sub> of unreacted and free lactic acid Table III. The signal at 1.48 ppm corresponded to -CH<sub>2</sub>- of fatty acid. The -CH<sub>2</sub>CO- of the fatty acid and its ester resonated at 2.18 ppm.

The -CH-O- signal region from lactic acid was more informative. The quartet at 4.03 ppm corresponded to unreacted lactic acid, while that at 4.19 ppm corresponded to hydroxyl terminal -CH-O- of the lactylates. The region from 4.85-5.20 ppm was the region of interest for this study as it corresponded to the fatty acid esters of lactic acid and lactic acids along with free lactic acids. This region can be considered as the finger print region for these esters. The samples showed increase in the areas of the -CH-O- quartets (when compared to those of the crystalline

**Table III**— $^1\text{H}$  NMR data on the fatty acid esters of lactic acid

| Chemical shift values<br>( $\delta$ , in ppm) ( $J$ , Hz) | Assignments                          | Chemical shift values<br>( $\delta$ , in ppm) ( $J$ , Hz) | Assignments                          |
|-----------------------------------------------------------|--------------------------------------|-----------------------------------------------------------|--------------------------------------|
| Stearoyl lactic acid                                      |                                      | Commercial sodium stearoyl lactic acid<br>(acidified)     |                                      |
| 0.85(t) (6.82)                                            | 18-CH <sub>3</sub> <sup>a</sup>      | 0.85 (t) (7.04)                                           | 18-CH <sub>3</sub> <sup>a</sup>      |
| 1.24(t)                                                   | 4-17 -CH <sub>2</sub> - <sup>a</sup> | 1.25 (t) (7.10)                                           | 4-17 -CH <sub>2</sub> - <sup>a</sup> |
| 1.42(d) (7.20)                                            | -CH <sub>3</sub> <sup>b</sup>        | 1.51 (d)                                                  | -CH <sub>3</sub> <sup>b</sup>        |
| 1.48(d)                                                   | 3-CH <sub>2</sub> - <sup>a</sup>     | 1.60 (d)                                                  | 3-CH <sub>2</sub> - <sup>a</sup>     |
| 2.18 (t) (7.35)                                           | 2-CH <sub>2</sub> CO- <sup>a</sup>   | 2.35 (t) (7.46)                                           | 2-CH <sub>2</sub> CO- <sup>a</sup>   |
| 4.03 (q) (7.20)                                           | -CH-O- <sup>c</sup>                  | 4.10(q)                                                   | -CH-O- <sup>c</sup>                  |
| 4.19 (q)                                                  | -CH-O- <sup>d</sup>                  | 4.35 (q)                                                  | -CH-O- <sup>d</sup>                  |
| 4.85-5.20(q)                                              | -CH-O- <sup>e</sup>                  | 5.09-5.35(q)(7.1)                                         | -CH-O- <sup>e</sup>                  |
| Palmitoyl lactic acid                                     |                                      | Lauroyl lactic acid                                       |                                      |
| 0.86 (t) (6.92)                                           | 16-CH <sub>3</sub> <sup>a</sup>      | 0.86 (t) (7.10)                                           | 12-CH <sub>3</sub> <sup>a</sup>      |
| 1.25 (t)                                                  | 4-15 -CH <sub>2</sub> - <sup>a</sup> | 1.25 (t)                                                  | 4-11 -CH <sub>2</sub> - <sup>a</sup> |
| 1.42 (d) (7.10)                                           | -CH <sub>3</sub> <sup>b</sup>        | 1.42 (d) (7.05)                                           | -CH <sub>3</sub> <sup>b</sup>        |
| 1.48 (d)                                                  | 3-CH <sub>2</sub> - <sup>a</sup>     | 1.48 (d)                                                  | 3-CH <sub>2</sub> - <sup>a</sup>     |
| 2.18 (t) (7.08)                                           | 2-CH <sub>2</sub> CO- <sup>a</sup>   | 2.18 (t) (7.12)                                           | 2-CH <sub>2</sub> CO- <sup>a</sup>   |
| 4.03 (q) (6.92)                                           | -CH-O- <sup>c</sup>                  | 4.03 (q) (7.05)                                           | -CH-O- <sup>c</sup>                  |
| 4.19 (q)                                                  | -CH-O- <sup>d</sup>                  | 4.19 (q)                                                  | -CH-O- <sup>d</sup>                  |
| 4.85-5.2(q)                                               | -CH-O- <sup>e</sup>                  | 4.85-5.2(q)                                               | -CH-O- <sup>e</sup>                  |

<sup>a</sup>-From free and esterified fatty acid; <sup>b</sup>-From free and esterified lactic acid; <sup>c</sup>- From free lactic acid; <sup>d</sup>-Hydroxyl terminal lactic acid of lactylates; <sup>e</sup>-Fatty acid esters of lactic and lactic acids along with oligomeric lactylates

lactic acid employed for reaction) indicating esterification. Actual amount of the ester formed was worked out from the areas of the -CH-O- signals at 4.85-5.20 ppm with respect to free lactic acid -CH-O- at 4.05 ppm. **Table IV** shows the proportions of esters present in the samples as deduced by  $^1\text{H}$  NMR analysis. Enzymatically prepared lauroyl, palmitoyl and stearoyl lactic acids showed 34.1 %, 22.2 and 26.3 %, ester formation, respectively.

The reaction mixture in chloroform was first washed with water to remove unreacted lactic acid and then washed with saturated solution of sodium bicarbonate to neutralize unreacted organic acid as well as residual lactic acid present in the sample. All the three layers (water, bicarbonate and chloroform) were evaporated and  $^1\text{H}$  NMR spectra were recorded for these samples. It was observed that both water (including bicarbonate) as well as chloroform layers contained good amounts of the esters. Free lactic acid along with organic acid esters of lactic and lactic acids were found to be predominantly present in water, while aqueous bicarbonate layer was found to contain very little stearic acid **Table IV**.

Comparative data of the extent of esterification as determined by titration method, NMR and HPLC are given in **Table IV**. NMR and HPLC proportions of ester formation showed good correlation. During the

course of the reaction, not only did fatty acids form esters with lactic and lactic acids, but lactic acid itself underwent esterification to form lactic acids. Hence, decrease in total acid content determined by titration method was always more than the actual amount of the fatty acid ester formed, which was determined accurately by HPLC and  $^1\text{H}$  NMR.

### $^{13}\text{C}$ NMR of organic acid esters of lactic acid

$^{13}\text{C}$  NMR spectra of reaction mixtures containing fatty acid esters of lactic and lactic acids were recorded on a 500 MHz NMR instrument operating at 125 MHz for carbon-13. **Table V** shows the chemical shift values of ester samples prepared by lipase catalysis and their assignments. For assigning the spectra, the labelling indicated in **Scheme II** was employed. The assignments were based on those of Gunstone *et al.*<sup>8</sup>, Bus *et al.*<sup>9</sup> and Rakoff *et al.*<sup>10</sup>. Some of the assignments are interchangeable.

$^{13}\text{C}$  NMR data of stearoyl, palmitoyl and lauroyl lactic acids are given in **Table V**. The signals corresponding to reacted and unreacted lauric acid were similar to that of palmitic acid **Table V**. The indication of ester formation could be observed from C-1 and C-2 signals of fatty acid and -CH-O- and -CO- signals of lactic/lactic acids. The C-2 signal at 34.6 ppm was associated with a less intense upfield signal

**Table IV**—Yields of lactic acid esters from Titration, HPLC and  $^1\text{H}$  NMR<sup>a</sup>

| Sample                                 | Ester Yield (%)<br>(Titration) | Free lactic acid (%) | Lactylate esters <sup>b</sup> (%) | Organic acid esters of lactic/lactylic acids (%) | Ester yield by HPLC |
|----------------------------------------|--------------------------------|----------------------|-----------------------------------|--------------------------------------------------|---------------------|
| Commercial lactic acid                 |                                | 64.1                 | 35.9                              |                                                  |                     |
| Crystalline lactic acid                |                                | 28.1                 | 71.9                              |                                                  |                     |
| Stearoyl lactic acid                   | 83.6                           | 40.9                 | 25.0                              | 34.1                                             | 38.2                |
| Stearoyl lactic acid–water layer       | 83.6                           | 85.5                 | 2.72                              | 10.8                                             |                     |
| Stearoyl lactic acid–bicarbonate layer | 83.6                           | -                    | -                                 | -                                                |                     |
| Stearoyl lactic acid–chloroform layer  | 83.6                           | 8.2                  | 21.3                              | 70.5                                             |                     |
| Palmitoyl lactic acid                  | 61.6                           | 44.4                 | 33.3                              | 22.2                                             | 24.9                |
| Lauroyl lactic acid                    | 43.7                           | 28.2                 | 45.6                              | 26.3                                             | 21.7                |

<sup>a</sup> Percentage of organic acid esters of lactic /lactylic acids were determined from the increase in the area of  $-\text{CH-O}-$  signals in the 4.9-5.20 ppm region in the spectra of samples with respect to that of crystalline lactic acid at 4.05 ppm; Error in NMR measurements was  $\pm 5\%$ , HPLC  $+5\%$  and Titration  $+5 - 10\%$ ; <sup>b</sup>Oligomers of lactic acid.

**Table V**— $^{13}\text{C}$  NMR data on organic acid esters of lactic acid<sup>a</sup>.

| Sample                                         | Chemical shift values( $\delta$ ppm)   |                 |                  |                                     |                          |                    |
|------------------------------------------------|----------------------------------------|-----------------|------------------|-------------------------------------|--------------------------|--------------------|
|                                                | $-\text{CH}_3$                         | $-\text{CH}_2-$ | $-\text{CH-O}-$  | $-\text{CO-}$<br>Ester <sup>f</sup> | $-\text{COOH}$<br>(free) |                    |
| Palmitic acid                                  | 14.7 <sup>b</sup>                      | 23.2            | C-14             |                                     |                          |                    |
|                                                |                                        | 25.6            | C-15             |                                     |                          |                    |
|                                                |                                        | 29.8-30.3       | C-4-C-13         |                                     |                          |                    |
|                                                |                                        | 32.5            | C-3              |                                     |                          |                    |
|                                                |                                        | 34.7            | C-2              |                                     |                          |                    |
| Lauroyl lactic acid                            | 14.7 <sup>b</sup><br>17.5 <sup>c</sup> | 23.2            | C-10             | 66.8 <sup>c</sup>                   | 172.7                    | 175.3 <sup>e</sup> |
|                                                |                                        | 25.5            | C-11             | 69.0 <sup>c</sup>                   | 175.0                    | 177.3 <sup>d</sup> |
|                                                |                                        | 29.7-30.2       | C-4-C-9          | 69.9 <sup>f</sup>                   |                          |                    |
|                                                |                                        | 32.4            | C-3              |                                     |                          |                    |
|                                                |                                        | 34.3            | C-2 <sup>g</sup> |                                     |                          |                    |
| Palmitoyl lactic acid                          | 14.7 <sup>b</sup><br>17.6 <sup>c</sup> | 23.2            | C-14             | 66.8 <sup>c</sup>                   | 172.7                    | 175.2 <sup>e</sup> |
|                                                |                                        | 25.6            | C-15             | 66.7-70.0 <sup>f</sup>              | 172.2                    | 177.3 <sup>d</sup> |
|                                                |                                        | 29.8-30.3       | C-4-C-13         |                                     |                          |                    |
|                                                |                                        | 32.5            | C-3              |                                     |                          |                    |
|                                                |                                        | 34.6            | C-2              |                                     |                          |                    |
| Stearoyl lactic acid                           | 14.7 <sup>b</sup><br>17.7 <sup>c</sup> | 23.2            | C-16             | 66.7 <sup>b</sup>                   | 172.7                    | 175.2 <sup>e</sup> |
|                                                |                                        | 25.6            | C-17             | 66.8 <sup>b</sup>                   |                          | 177.3 <sup>d</sup> |
|                                                |                                        | 29.8-30.3       | C-4-C-15         | 69.3 <sup>f</sup>                   |                          |                    |
|                                                |                                        | 32.5            | C-3              |                                     |                          |                    |
|                                                |                                        | 34.7            | C-2              |                                     |                          |                    |
| Commercial stearoyl lactic acid<br>(acidified) | 14.7 <sup>b</sup><br>17.4 <sup>c</sup> | 23.3            | C-16             | 65.7 <sup>c</sup>                   | 173.9                    | 180.7 <sup>e</sup> |
|                                                |                                        | 25.3            | C-15             | 68.6 <sup>f</sup>                   |                          | 177.0 <sup>d</sup> |
|                                                |                                        | 25.4-30.6       | C-4-C-14         |                                     |                          |                    |
|                                                |                                        | 32.5            | C-3              |                                     |                          |                    |
|                                                |                                        | 34.5            | C-2 <sup>f</sup> |                                     |                          |                    |
|                                                |                                        | 34.6            | C-2              |                                     |                          |                    |

<sup>a</sup> Assignments are based on Bus *et al.* (1977); Gunstone *et al.* (1977) and Rakoff and Emken (1982). Some of the assignments are interchangeable. <sup>b</sup>From free and esterified fatty acids; <sup>c</sup>From free and esterified lactic/lactylic acids; <sup>d</sup> From free lactic acid; <sup>e</sup> Free fatty acid; <sup>f</sup>Fatty acid esters of lactic/lactylic acids

corresponding probably to the ester (34.3 ppm). The signal at 66.8 ppm, corresponded to -CH-O- of unreacted lactic acid, whereas the downfield ones around 69.0 ppm corresponded to -CH-O- from fatty acid esters of lactic and lactic acids. In the carbonyl carbon region, two downfield shifts observed at 177.3 and 175.3 ppm corresponded to free fatty acid and lactic acid, respectively. The ester -CO- signal could be observed in the upfield region at 172.7 ppm in all the cases.

Both  $^1\text{H}$  and  $^{13}\text{C}$  NMR data also showed that the enzymatically prepared samples showed all the NMR characteristics of the commercial samples, which is very important from the technology viewpoint.

### Experimental Section

**Lipases.** Porcine pancreas lipase (triacylglycerol hydrolase E. C. 3.1.1.3 – PPL), a Type II (Steapsin), a crude enzyme preparation, was purchased from Sigma Chemical Co, St. Louis, USA. Lipozyme IM-20, a *Rhizomucor miehei* lipase, immobilized on to a duolite weak anion exchange resin was purchased from Novo Nordisk, Denmark.

Esterification activities of lipases were determined by an esterification method<sup>11</sup> otein was estimated by Lowry's method<sup>12</sup>. Esterification activities of PPL and Lipozyme IM20 were 0.05, 0.49  $\mu\text{mol}/\text{min}.\text{mg}$  enzyme and 0.16, 26.9  $\mu\text{mol}/\text{min}.\text{mg}$  protein (specific activity), respectively.

**Chemicals.** Acetic, propionic, butyric, isobutyric, octanoic, lauric, palmitic and stearic acids were purchased from Sd. Fine Chemicals Ltd., India. Valeric and isovaleric acids were purchased from Sigma-Aldrich Chemical Company. Crystalline lactic acid employed for the reactions was prepared from commercial, 88% pure lactic acid (from Qualigens Ltd., India) by azeotropic reflux with benzene. All the solvents employed for the reactions were procured from Sisco Research Laboratories Ltd., India, and were distilled once before use.

**Esterification procedures.** Esterification reactions were carried out at both shake-flask<sup>6</sup> as well at bench-scale level<sup>5,13</sup> and the products formation were monitored by a back titration procedure<sup>5</sup>. After the reaction, the products from the reaction mixture were worked out by filtering the insoluble lipase and then evaporating the solvent to get a mixture containing unreacted fatty acid, lactic acid and lactic acids, which were taken for estimation by HPLC and NMR. Since, even chemical preparations lead to a mixture of

compounds, no attempt was made to purify the enzymatic reaction mixtures.

### HPLC

A Shimadzu HPLC instrument connected to a reverse phase  $\text{C}_{18}$  column (Shimpack-ODS) was used. Solvent system consisting of acetonitrile: orthophosphoric acid, 5 mM [90:10] at a flow rate of 0.8 ml/min was employed as an isocratic mobile phase. All the samples were prepared in the mobile phase. A UV detector operating at a wavelength of 205 nm was used for monitoring the elution.

### NMR Spectroscopy

Brüker DRX 500 and AMX 400 instruments operating at 500 and 400 MHz respectively, for  $^1\text{H}$ , fitted with Spectrospin magnet and Aspect 2000 computer were used to record the spectra.

$^1\text{H}$  NMR spectra were recorded in  $\text{DMSO}-d_6$  or  $\text{CDCl}_3$  with about 20-30 mg of the sample dissolved in 0.5 mL of the solvent. Samples were field-frequency locked to deuterium. About 50-200 scans were accumulated with a recycle period of 2-3 seconds to obtain good spectra. The spectra were recorded at 20°C with TMS as internal standard for measuring the chemical shift values to within  $\pm 0.01$  ppm. Typically, a region of 0-15 ppm was scanned for each spectrum.

$^{13}\text{C}$  NMR spectra were recorded on the DRX 500 and AMX 400 NMR instruments operating at 125 Hz and 100 MHz, respectively. A region from 0-250 ppm was scanned and 500-2000 scans were accumulated for each spectrum. All signals were referenced to TMS to within  $\pm 0.1$  ppm. Spectra were recorded in  $\text{DMSO}-d_6$  or  $\text{CDCl}_3$  with about 50-100 mg of the sample dissolved in 0.5 mL of the solvent. Proton-noise decoupled spectra were obtained.

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