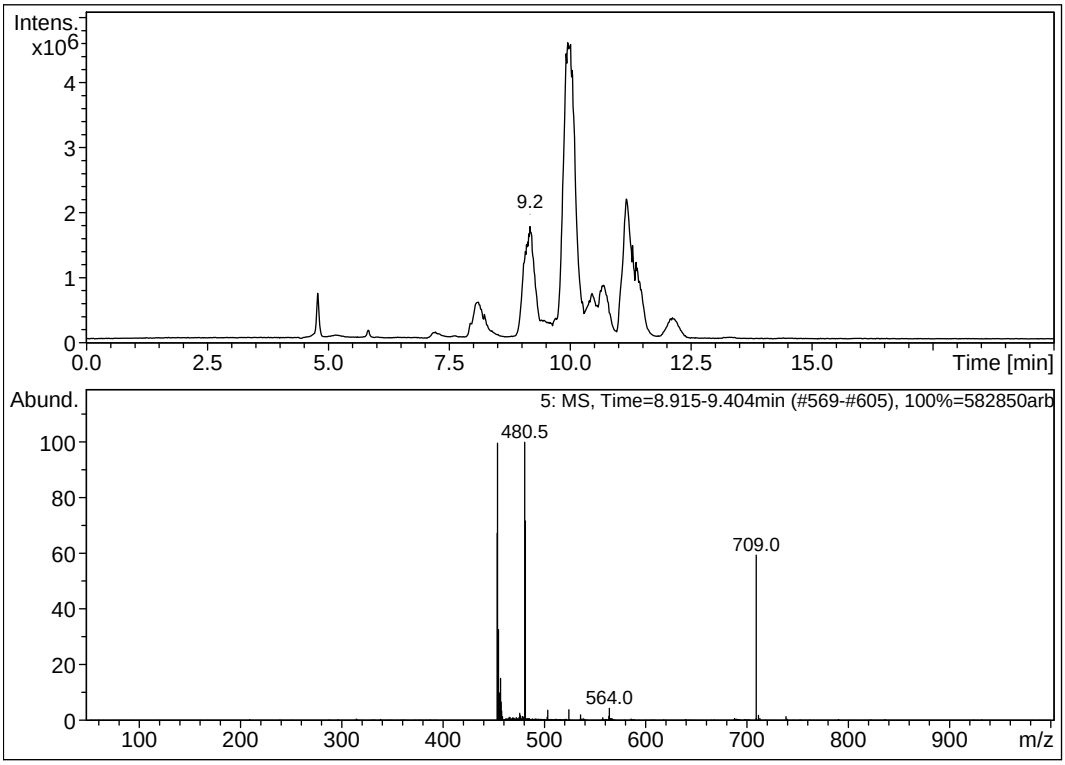
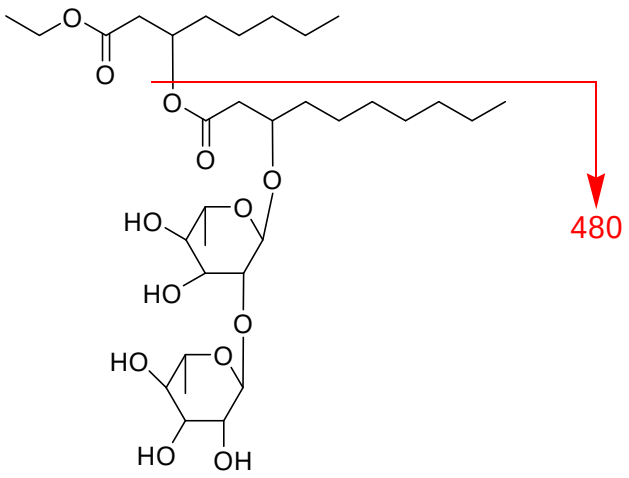
Supporting information

Part 1. The mass spectra corresponding to signals 1-4 in Figure 3

Signal 1

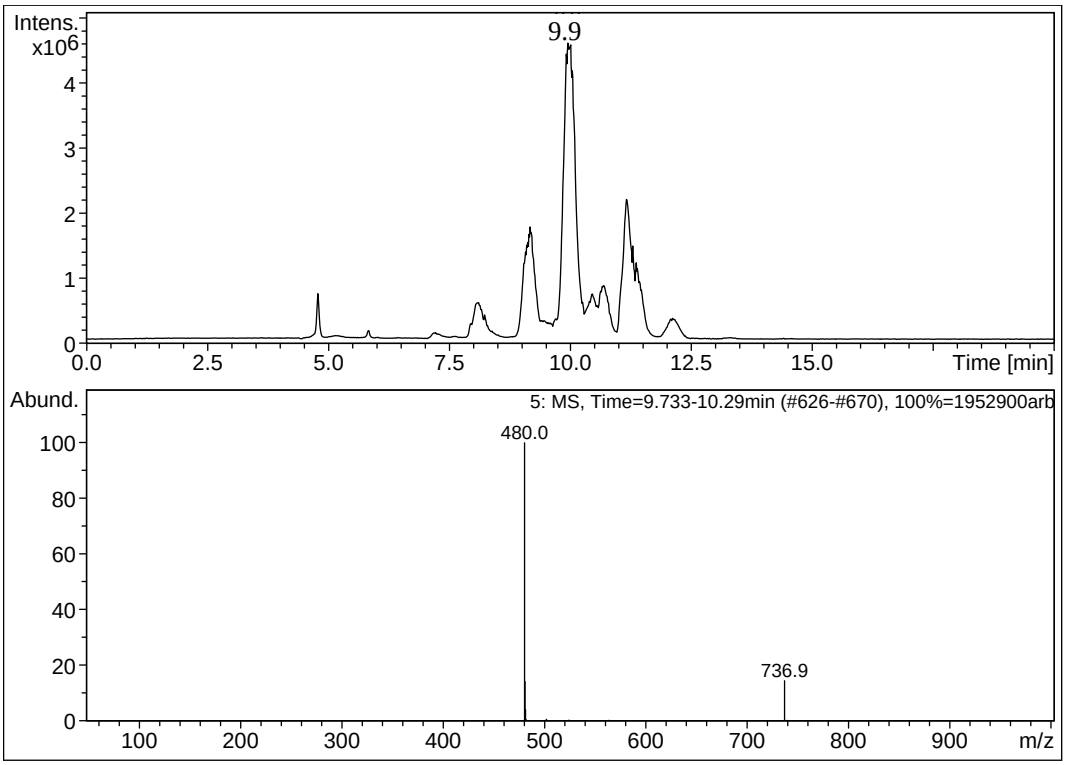


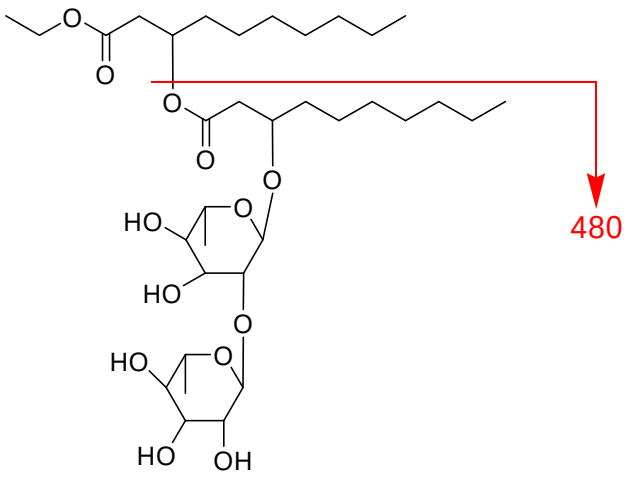


Ethylated R-R-C10-C8, with molecular weight of 650

Signal m/z = 709 (= 650 + 59), corresponding to the adduct anion of [molecule + CH_3COO^-] formed due to the presence of ammonium acetate in the mobile phase

Signal 2

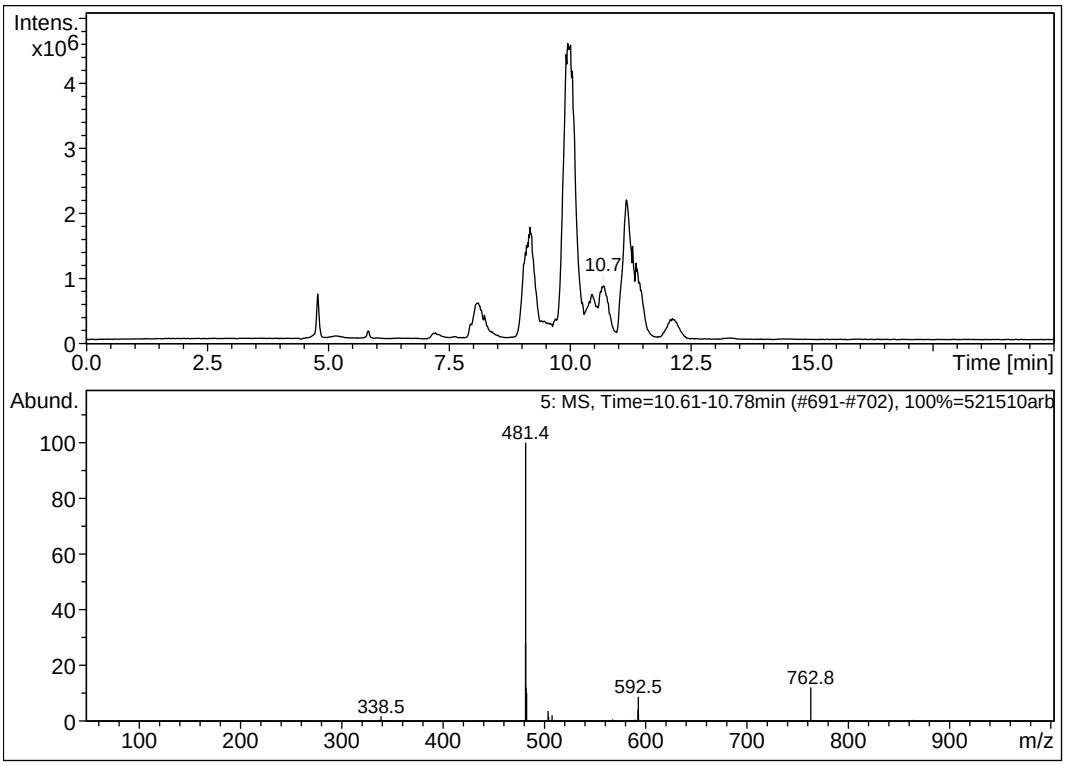


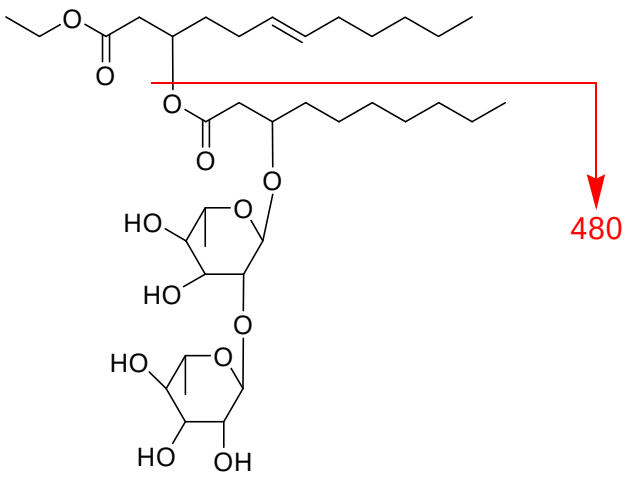


Ethylated R-R-C10-C10, with molecular weight of 678

Signal m/z = 737 (= 678 + 59), corresponding to the adduct anion of [molecule + CH_3COO^-] formed due to the presence of ammonium acetate in the mobile phase

Signal 3

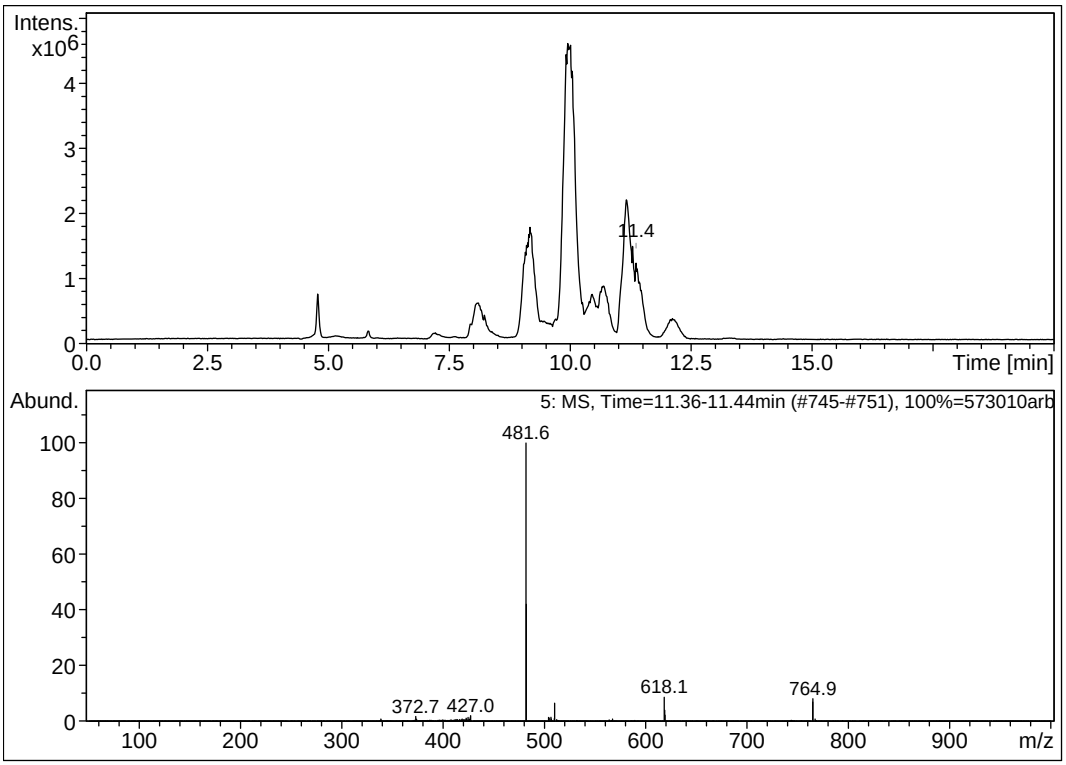


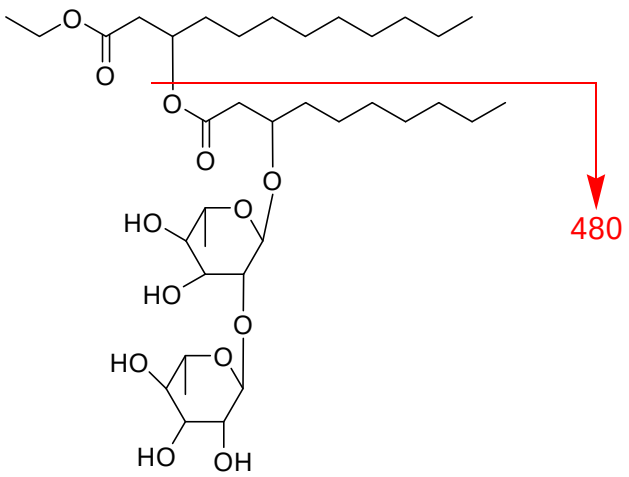


Ethylated R-R-C10-C12:1, with molecular weight of 704

Signal m/z = 763 (= 704 + 59), corresponding to the adduct anion of [molecule + CH_3COO^-] formed due to the presence of ammonium acetate in the mobile phase

Signal 4



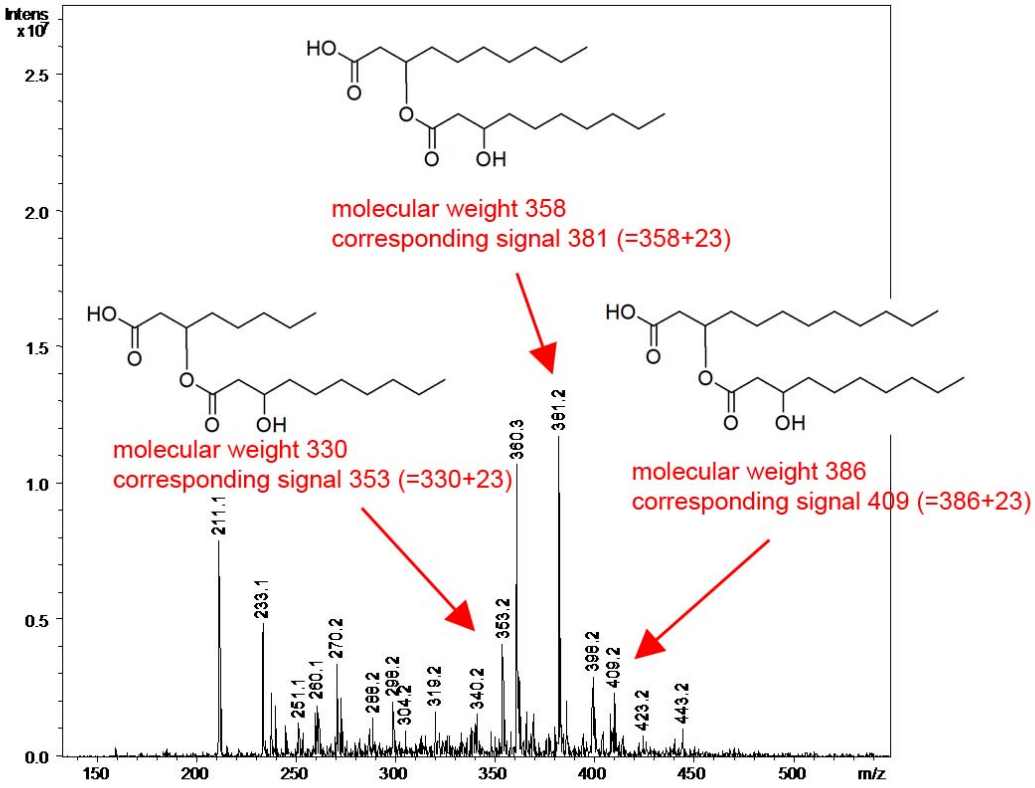


Ethylated R-R-C10-C12, with molecular weight of 706

Signal m/z = 765 (= 706 + 59), corresponding to the adduct anion of [molecule + CH_3COO^-] formed due to the presence of ammonium acetate in the mobile phase

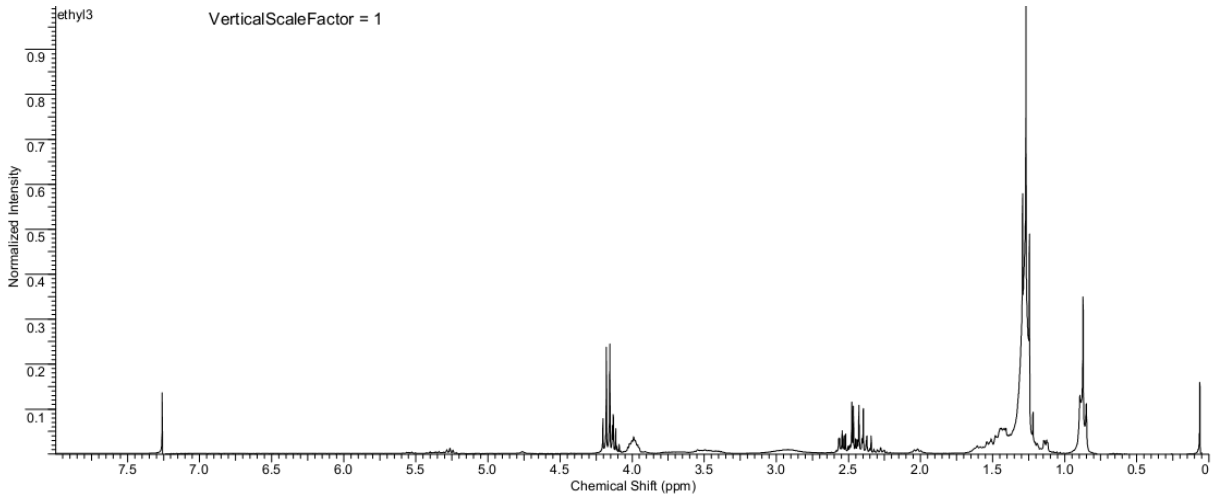
Part 2. The free diacids obtained from rhamnolipids

(A) Result of mass analysis in the positive mode for the product mixture sampled at the end of the two-step reactions: (1) formation of ethyl diacids from RhL-3 and (2) hydrolysis of ethyl to free diacids. Mainly free diacids are seen in the mass spectrum below.

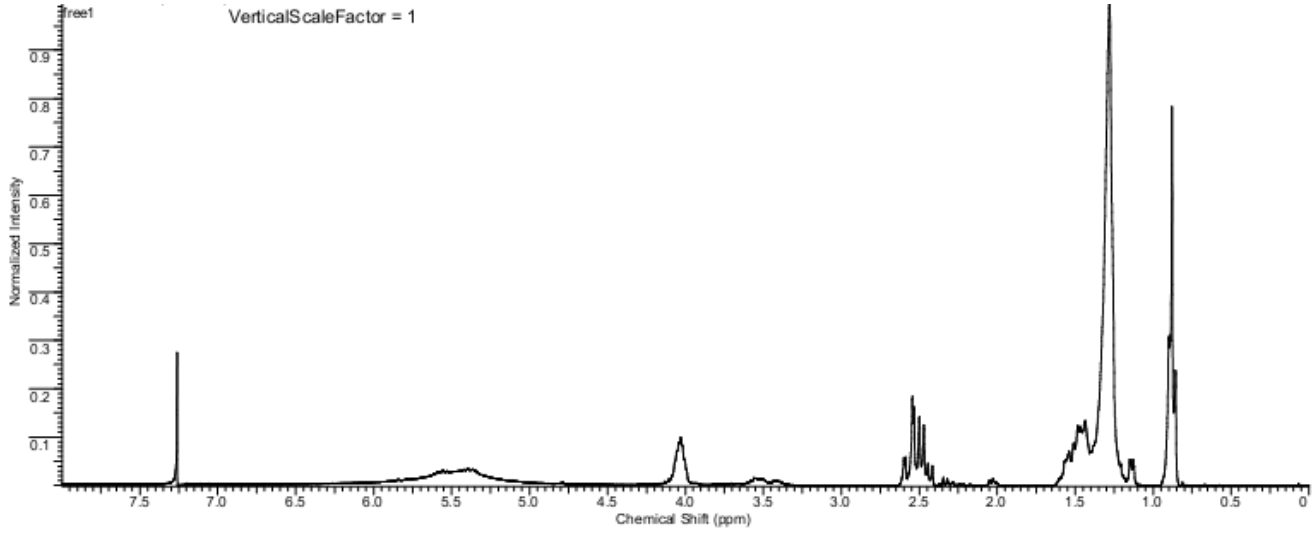


(B) Samples from the hydrolysis reaction of ethyl diacids to free diacids were also detected with ^{1}H NMR analysis. The results for samples taken before and after the reaction are shown here. The peak at 4.15 ppm in spectrum (1) corresponds to the ethyl group, which disappears after the hydrolysis as shown in spectrum (2). Other peaks remain unchanged.

(1) Sample before hydrolysis, containing ethyl diacids:



(2) Sample after hydrolysis, containing free diacids:

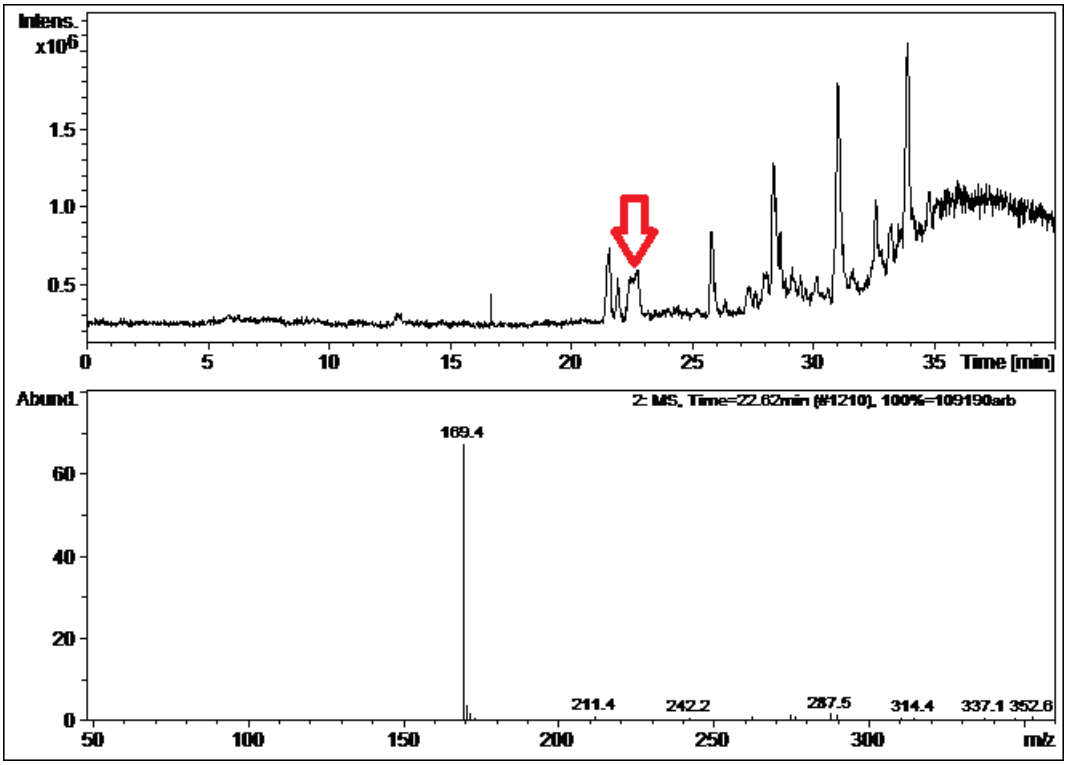


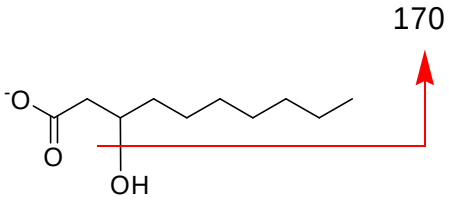
The same sodium hydroxide-catalyzed hydrolysis was also applied to RhL-3 directly (without first forming the ethyl diacids from RhL-3). Results of the HPLC-MS analysis show that the ester group between the two β-hydroxyl fatty acids in rhamnolipids was not hydrolyzed (data not shown).

Part 3. Hydroxyl fatty acids observed in the synthesis of RhL-2 and RhL-4 from RhL-1 and RhL-3

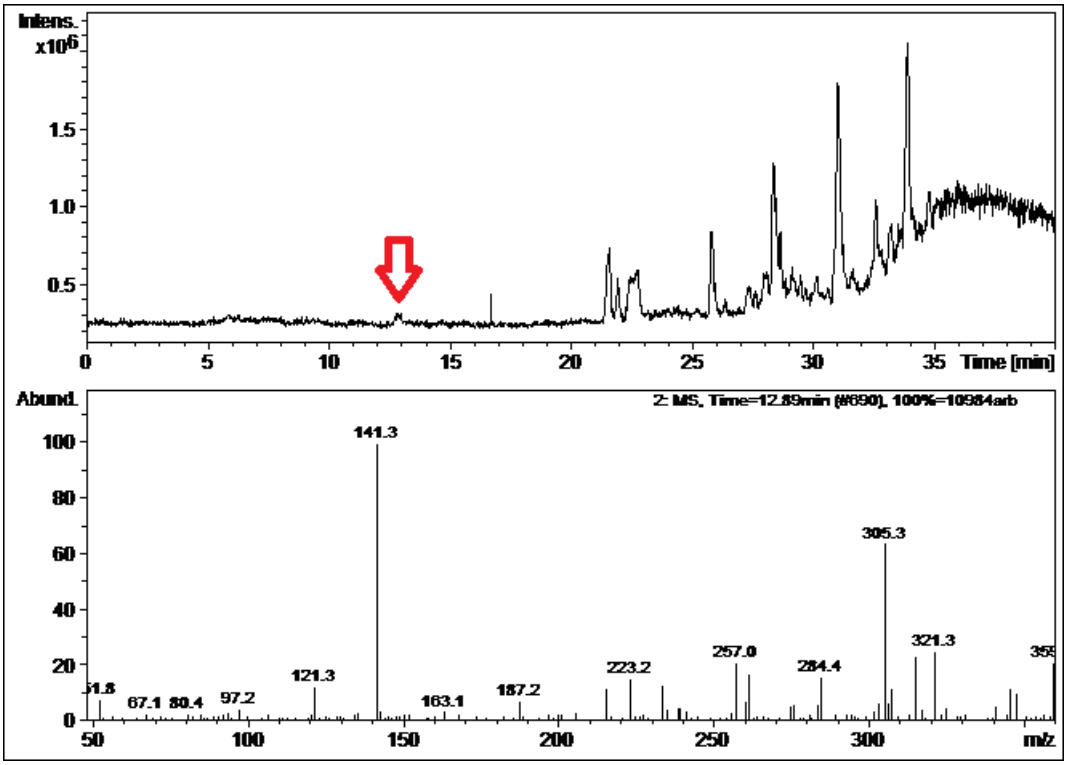
About 100 mg of the final reaction mixture was added to a silica column (2.5 cm x 30 cm). The eluent used was dichloromethane/methanol/water (100/30/5). Thirty fractions were collected, 10 ml for each fraction. The following HPLC-MS spectra are obtained for a sample of fraction 7. The signals for hydroxyl fatty acids (C8 and C10) are marked with arrows. The HPLC mobile phase used was a varying mixture of methanol and 4 mM aqueous ammonium acetate, according to the following method of segment gradients: (1) a 5-min isocratic hold at 40% methanol and 60% water; (2) a 20-min gradient to increase the methanol content from 40% to 95%; (3) a 10-min isocratic hold at 95% methanol and 5% water; (4) a 15-min gradient to decrease the methanol content from 95% to 40%; and (5) a final 5-min isocratic hold at 40% methanol and 60% water. The analysis lasted for a total of 55 min. The MS analysis was made in the negative mode. Besides the hydroxyl fatty acids, other signals were also observed. Although these signals have not yet been studied in detail, they are believed to correspond to fatty acids.

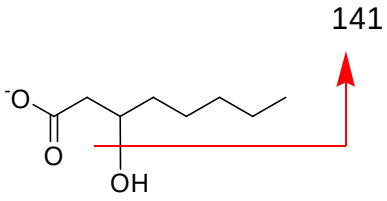
C10:


Intens.
x10^6
1.5
1.0
0.5
0
5
10
15
20
25
30
35
Time [min]
Abund.
2: MS, Time=22.62min (#1210), 100%=109190arb
169.4
60
40
20
0
211.4
242.2
287.5
314.4
337.1
352.6
50
100
150
200
250
300
m/z


170
O
O
OH

C8:


Intens.
x10^6
1.5
1.0
0.5
0
5
10
15
20
25
30
35
Time [min]
Abund.
2: MS, Time=12.89min (#690), 100%=10984arb
100
80
60
40
20
0
141.3
305.3
321.3
257.0
284.4
223.2
121.3
187.2
163.1
97.2
67.1
80.4
50
100
150
200
250
300
m/z


141
O
O
OH