SEVEN NEW MONOGALACTOSYL DIACYLGLYCEROLS ISOLATED FROM THE AXENIC CYANOBACTERIUM *PHORMIDIUM TENUE*[1)]

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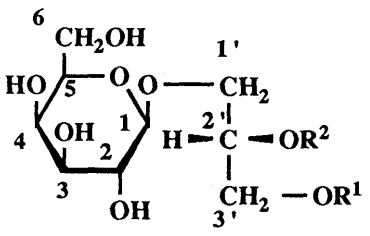
Seven new galactolipids (**2** to **8**) were isolated from the cultured cyanobacterium *P. tenue* as substances causing lysis of this alga. The structures of these galactolipids were determined by physicochemical evidence and enzymatic hydrolysis using lipase. Those galactolipids which contained unsaturated fatty acids were more potently autolytic.

KEYWORDS axenic; cyanobacterium; *Phormidium tenue*; galactolipid; monogalactosyl diacylglycerol; enzymatic hydrolysis; lipase; lysis

In a previous paper,[2)] we reported that an autolytic substance, isolated from *P. tenue*, is a mixture of some saturated and unsaturated fatty acids. Linoleic and linolenic acids are more potently autolytic than saturated ones. In continuing the investigation, we have now isolated nine galactolipids (**1** to **9**) whose autolytic activity was weaker and slower than that of the previous mixture. Here, we report on their structures and autolytic activity. The acetone extract (196 mg) of the lyophilized alga (6.5 g), obtained as described before,[2)] was submitted to silica gel column chromatography ($CHCl_3$-MeOH=10:1). The eluate (96 mg), showing a single spot on thin-layer chromatography, was further purified by HPLC (Develosil ODS-5 and Develosil ODS-K5, MeOH:acetone:H_2O=60:40:5) to afford nine galactolipids (**1** to **9**), one of which (**9**) was a mixture of two compounds.

A major galactolipid (**2**), $[\alpha]_D$ -2.7° ($CHCl_3$), IR($CHCl_3$):3500, 1730 cm^{-1}, afforded a quasimolecular ion peak at m/z 747$(M+Na)^+$ in FAB-MS. The ^1H NMR and the ^{13}C NMR spectra of **2** showed signals characteristic of a galactolipid (Table I). Treatment of **2** with 10% NaOMe-MeOH gave monogalactosyl glycerol (**10**), methyl myristate and methyl linolenate. Comparing the ^1H and ^{13}C NMR spectra of **2** with those of **10** showed that in **2** the proton signals due to 2'-H and 3'-H_2 of the glycerol moiety appeared in a lower field, while the carbon signal due to the C-1' of **2** appeared in a higher field. The signals assignable to C-2' and C-3' of **2** and **10** showed similar chemical shifts.[3)] Thus, the fatty acid residues would attach to C-2' and C-3' in the glycerol moiety. The monogalactosyl glycerol (**10**), $[\alpha]_D$ -8° (H_2O) was identical in all respects with (2'R)-1-*O*-glyceryl β-D-galactopyranoside.[4)] To determine the location of the two fatty acid residues in **2**, we attempted regioselective deacylation by lipase. Enzymatic hydrolysis using Lipase type XI (from *Rhizopus arrhizus*, Sigma Co., Ltd.) in the presence of Triton X-100 in boric acid-borax buffer (pH 7.7) at 37 °C for 1.5 h gave 3'-*O*-deacylated galactolipid (**2a**)[5)] and linolenic acid quantitatively. As shown in Table I, the ^1H NMR spectrum of **2a** exhibited the signals due to 3'-H_2 in a higher field than those in **2**. In addition, alkaline (NaOMe-MeOH) treatment of **2a** liberated methyl myristate. Based on these findings, the structure of the galactolipid (**2**) was determined as (2'S)-2'-*O*-(tetradecanoyl)-3'-*O*-(9*Z*, 12*Z*, 15*Z*-octadecatrienoyl)-glyceryl-β-D-galactopyranoside.



1: $R^1=R^2$=linolenoyl
2: R^1=linolenoyl, R^2=myristoyl
2a: R^1=H, R^2=myristoyl
3: R^1=linolenoyl, R^2=palmitelaidoyl
3a: R^1=H, R^2=palmitelaidoyl
4: R^1=palmitoleoyl, R^2=myristoyl
5: R^1=linoleoyl, R^2=myristoyl
6: R^1=oleoyl, R^2=myristoyl
7: R^1=linoleoyl, R^2=palmitoyl
8: R^1=palmitoyl, R^2=myristoyl
9: a: R^1=linolenoyl, R^2=palmtoyl
b: R^1=linoleoyl, R^2=palmitelaidoyl
(a:b=76:24)
10: $R^1=R^2$=H

Table I. ^{1}H NMR Data for **2**, **2a**, **3**, and **10**[a)]

H	2	2a	3	10
1	4.84 (d, J=7.7)	4.85 (d, J=7.7)	4.84 (d, J=7.7)	4.91 (d, J=7.7)
2	4.46[b)]	4.45 (dd, J=7.7, 9.5)	4.46[b)]	4.52 (dd, J=7.7, 9.3)
3	4.16 (dd, J=3.4, 9.4)	4.13 (dd, J=3.4, 9.5)	4.16 (dd, J=3.3, 9.3)	4.17 (dd, J=3.3, 9.3)
4	4.57 (d, J=3.4)	4.53 (d, J=3.4)	4.57 (d, J=3.3)	4.56 (d, J=3.3)
5	4.08 (dd, J=5.7, 5.7)	4.04 (dd, J=6.5, 6.5)	4.09 (dd, J=5.7, 5.7)	4.08 (dd, J=5.3, 6.6)
6	4.42[b)]	4.42 (d, J=6.5)[c)]	4.42[b)]	4.45[b)]
		4.41 (d, J=6.5)		
1'	4.40 (dd, J=5.3, 10.8)	4.49 (dd, J=4.8, 11.7)	4.40 (dd, J=4.8, 10.9)	4.45[b)]
	4.09 (dd, J=5.0, 10.8)	4.24 (dd, J=5.3, 11.7)	4.10 (dd, J=5.4, 10.9)	4.27 (dd, J=3.8, 9.7)
2'	5.71 (m)	5.61 (m)	5.70 (m)	4.45[b)]
3'	4.72 (dd, J=3.1, 10.8)	4.20 (dd, J=5.5, 10.6)	4.70 (dd, J=3.3, 11.9)	4.14 (d, J=4.9)[c)]
	4.54 (dd, J=6.2, 10.8)	4.14 (dd, J=5.0,10.6)	4.54 (dd, J=3.3, 11.9)	4.13 (d, J=5.5)

a)All compounds were measured in pyridine-d_5 (treated with 1 drop of D_2O) at 400 MHz and the assignments were based on decoupling experiments. b)These signals overlapped within the same vertical column. c)They had fairly close chemical shifts, therefore they had no coupling constants due to geminal protons. On irradiation of the 5-H proton signal in **2a** and 2'-H proton signal in **10**, these signals appeared as singlet.

The galactolipid (**3**), $[\alpha]_D$ -3.4° ($CHCl_3$), IR($CHCl_3$): 3500, 1730 cm^{-1}, gave a quasimolecular ion peak at m/z 773$(M+Na)^+$ in FAB-MS. The ^{1}H NMR spectrum of **3** closely resembled that of **2** except for the signals due to the fatty acid residues. Enzymatic hydrolysis of **3** gave linolenic acid and 3'-*O*-deacylated galactolipid (**3a**), which liberated a methyl ester when treated with NaOMe-MeOH. Although this methyl ester was found to have a molecular formula of $C_{17}H_{32}O_2$, it differed from methyl palmitoleate. So we applied the piconyl method to determine the location of its double bond.[6)] Treatment of the fatty acid, prepared on enzymatic hydrolysis and subsequent alkaline treatment (KOH-MeOH), with 2-pyridylcarbinol in CH_3CN furnished the piconyl ester which was subjected to GC-MS analysis. Its mass spectrum showed that the double bond was located between C_9 and C_{10} from its fragmentation pattern. Thus, the fatty acid was determined to be palmitelaidic acid, a geometrical isomer of palmitoleic acid. Consequently, the structure of **3** was found to be (2'S)-2'-*O*-(9*E*-hexadecenoyl)-3'-*O*-(9Z,12Z,15Z-octadecatrienoyl)-β–D-galactopyranoside. The chemical structures of the other galactolipids were also characterized in the same manner. Among the nine monogalactosyl diacylglycerols isolated from *P. tenue*, **1** was identified as the known compound,[7)] and **9** was shown to be a mixture of two galactolipids the separation of which is still unsuccessful. Seven galactolipids (**2** to **8**)[8)] were first isolated and their chemical structures were characterized including the positional determination of the fatty acid residues.

Table II. Minimum Concentrations of Each Monogalactosyl Diacylglycerol for Lysis of *P. tenue*

Compound	ppm	Compound	ppm
1	25	6	>100
2	10	7	25
3	50	8	>100
4	50	9	50
5	50	10	>100

As described above, we demonstrated that unsaturated fatty acids such as linoleic and linolenic acids were autolytic substances, but saturated ones such as myristic and palmitic acids did not lyse *P. tenue*. This indicates that the fatty acids attached to the glycerol moiety in the galactolipids cause the difference in the autolysis of this alga. Therefore, we determined the autolytic activity of each galactolipid. Bioassay was carried out by the method reported previously.[2)] The minimum concentrations causing lysis of *P. tenue* are shown in Table II.

The galactolipids containing unsaturated fatty acid residues lysed the alga in lower concentrations, while monogalactosyl glycerol (**10**) caused no lysis even at 100 ppm.

It is noted that the galactolipids (**3**, **4**, **5**, **7**) with myristic acid are rare in nature, and **3** is the first example to our knowledge of the monogalactosyl diacylglycerol containing palmitelaidic acid in a molecule, although they are widely distributed in the plant kingdom. Thus, it would be of interest to study their biosynthesis and biological activities.[4,9] We found that a mixture of fatty acids was the autolytic substance, which suggested that the lysis of *P. tenue* occurred due to the fatty acids liberated from galactolipids by esterase in the alga. The mechanism of the lysis of the alga by monogalactosyl diacylglycerols is currently under investigation.

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3) The ^{13}C NMR data for **2** (100 MHz, CD_3OD, δc):105.4(C-1), 72.4(C-2), 74.9(C-3), 70.2(C-4), 76.8(C-5), 62.5(C-6), 68.7(C-1'), 71.8(C-2'), 64.0(C-3'); The ^{13}C NMR data for **10**(100 MHz, CD_3OD, δc):105.3(C-1), 72.6(C-2), 74.9(C-3), 70.4(C-4), 76.8(C-5), 62.6(C-6), 72.1(C-1'), 72.2(C-2'), 64.1(C-3').

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8) **2**:^1H NMR(Py.-d_5, δ, 400 MHz):0.88(3H, t, J=6.8), 0.96(3H, t, J=7.5), 2.41(4H, m), 2.95(4H, m), 5.42-5.56(6H, m). **3**:^1H NMR(Py.-d_5, δ, 400 MHz):0.88(3H, t, J=6.8), 0.96(3H, t, J=7.5), 2.36(2H, t, J=7.4), 2.49(2H, br s), 2.94(4H, m), 5.42-5.56(8H, m). **4**:$[\alpha]_D$ -2.8°($CHCl_3$); IR($CHCl_3$, cm^{-1}):3500, 1730; FAB-MS(m/z):723$(M+Na)^+$; ^1H NMR(Py.-d_5, δ, 400 MHz): 0.88(6H, m), 2.39(4H, m), 4.06-4.14(2H, m, 5-H, 1'-H), 4.17(1H, dd, J=3.5, 9.4, 3-H), 4.40(1H, dd, J=5.4, 10.9, 1'-H), 4.43-4.50(3H, m, 2-H, 6-H_2), 4.52-4.59(2H, m, 4-H, 3'-H), 4.72(1H, dd, J=3.3, 11.9, 3'-H), 4.85(1H, d, J=7.7, 1-H), 5.52(2H, m), 5.70(1H, m, 2'-H). **5**:$[\alpha]_D$ -2.3°($CHCl_3$); IR($CHCl_3$, cm^{-1}):3500, 1735; FAB-MS(m/z):749 $(M+Na)^+$; ^1H NMR(Py.-d_5, δ, 400 MHz):0.87(3H, t, J=6.5), 0.88(3H, t, J=6.8), 2.40(4H, m), 2.93(2H, dd, J=5.6, 5.8), 4.05-4.13(2H, m, 5-H, 1'-H), 4.17(1H, dd, J=3.4, 9.5, 3-H), 4.40(1H, dd, J=5.4, 11.0, 1'-H), 4.43-4.50(3H, m, 2-H, 6-H_2), 4.52-4.60(2H, m, 4-H, 3'-H), 4.72(1H, dd, J=3.4, 12.0, 3'-H), 4.85(1H, d, J=7.8, 1-H), 5.52(4H, m), 5.70(1H, m, 2'-H). **6**:$[\alpha]_D$ -2.5°($CHCl_3$); IR($CHCl_3$, cm^{-1}):3520, 1730; FAB-MS(m/z):751$(M+Na)^+$; ^1H NMR(Py.-d_5, δ, 400 MHz): 0.89(6H, m), 2.40(4H, m), 4.05-4.12(2H, m, 5-H, 1'-H), 4.17(1H, dd, J=3.3, 9.5, 3-H), 4.40(1H, dd, J=5.4, 10.8, 1'-H), 4.42-4.50(3H, m, 2-H, 6-H_2), 4.52-4.60(2H, m, 4-H, 3'-H), 4.73(1H, dd, J=3.1, 11.9, 3'-H), 4.85(1H, d, J=7.7, 1-H), 5.51(2H, m), 5.72(1H, m, 2'-H). **7**:$[\alpha]_D$ -3.4°($CHCl_3$); IR($CHCl_3$, cm^{-1}):3530, 1735; FAB-MS(m/z):777$(M+Na)^+$; ^1H NMR(Py.-d_5, δ, 400 MHz):0.88(6H, m), 2.40(4H, m), 2.93(2H, t-like), 4.06-4.13(2H, m, 5-H, 1'-H), 4.17(1H, dd, J=3.1, 9.5, 3-H), 4.40(1H, dd, J=5.3, 10.8, 1'-H), 4.43-4.51(3H, m, 2-H, 6-H_2), 4.53-4.59(2H, m, 4-H, 3'-H), 4.73(1H, dd, J=3.1, 11.7, 3'-H), 4.85(1H, d, J=7.5, 1-H), 5.51(4H, m), 5.70(1H, m, 2'-H). **8**:$[\alpha]_D$ -2.8°($CHCl_3$); IR($CHCl_3$, cm^{-1}): 3530, 1735; FAB-MS(m/z):725$(M+Na)^+$; ^1H NMR(Py.-d_5, δ, 400 MHz):0.89 (6H, m), 2.39(4H, m), 4.07-4.13(2H, m, 5-H, 1'-H), 4.17(1H, dd, J=3.3, 9.5, 3-H), 4.40(1H, dd, J=5.5, 11.2, 1'-H), 4.43-4.52(3H, m, 2-H, 6-H_2), 4.54-4.60(2H, m, 4-H, 3'-H), 4.73(1H, dd, J=3.1, 11.9, 3'-H), 4.85(1H, d, J=7.9, 1-H), 5.70(1H, m, 2'-H).

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