phonate reagent (3) activated by benzo-1,2,5-thiadiazolidine 1,1-dioxide which play an essential role in activating the carboxyl group for β-lactam formation as the new bifunctional condensing reagent. Further utility of the reagent 3 for the formation of esters, thioesters, and peptides is being explored.

Experimental

Melting points were determined with Buchi 510 apparatus and were uncorrected. Thin-layer chromatography (TLC) was performed on Silica gel 60 F254 (Merck) plates, and spots were detected by ultraviolet (UV) irradiation. ^{1}H NMR spectra were measured with a tetramethylsilane (TMS) in $CDCl_3$.

Preparation of N,N-bis[diethoxyphosphinyl]benzo-1,2,5-thiadiazolidine 1,1-dioxide (3). A mixture of benzo-1,2,5-thiadiazolidine 1,1-dioxide (6.80 g, 0.04 mol) and triethylamine (8.10 g, 0.08 mol) in dichloromethane (150 mL) was stirred at room temperature and a solution of diethyl chlorophosphate (13.80 g 0.08 mol) in dichloromethane (20 mL) was added dropwise. Stirring was continued for 3 h. The mixture was evaporated and the residue was extracted with chloroform. The organic layer was washed successively with 5% $NaHCO_3$ (100 mL), brine (200 mL) and dried with Na_2SO_4. The solvent was removed under reduced pressure and the residue was crystallized from chloroform-hexane. Yield 15.96 g (90%), mp 120-122 ℃; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.27 (t, 12H, J=7.1 Hz), 4.23 (q, 8H, J=7.1 Hz), 7.02-7.36 (m, 4H).

The typical experimental procedure for the reaction of β-amino acid with N,N-bis[diethoxyphosphinyl]benzo-1,2,5-thiadiazolidine 1,1-dioxide; To a mixture of 3-benzylaminobutanoic acid (289 mg, 1.5 mmol) and N,N-bis[diethoxyphosphinyl]benzo-1,2,5-thiazolidine 1,1-dioxide (400 mg, 0.9 mmol) in acetonitrile (150 mL) at room temperature. After being stirred at 80 ℃ for 10 h, the reaction mixture was concentrated under reduced pressure and the residue was passed through a short silica gel column using ether and chloroform (2 : 1) to yield 1-benzyl-4-methylazetidine-2-one (153 mg, 88%).

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Spectral Evidence of 1,2-Linkage in Antifungal Rhamnolipid Produced by *Pseudomonas aeruginosa*

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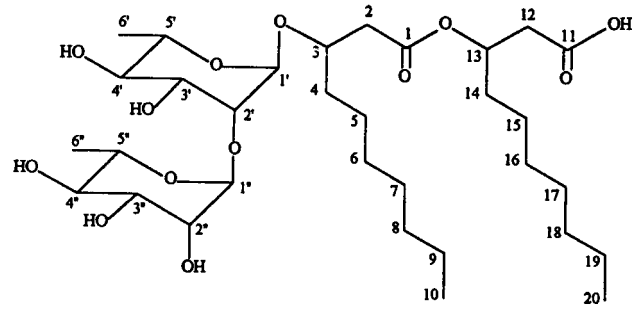
Biological efficacy of many antagonistic microorganisms may be mainly due to the formation of microbial metabolites acting as antibiotics. We reported the characterization of five fungicidal 4-quinolinones (PSC-A, B, C, D, E), which also showed red-pepper plant growth promoting effect, from *Pseudomonas cepacia* strain PC-II active against Phytophthora blight of red-pepper.[1,2] Recently, we isolated *Pseudomonas aeruginosa* strain B5 from pepper-growing soil,[3] which was highly antagonistic to *Phytophthora capsici* which causes foliar and stem blight of pepper (*Capsicum annuum* L.).[4] Antifungal substances P1, P2, and P3, which were inhibitory against some plant pathogenic fungi, were successfully purified from *P. aeruginosa* cultures.[3] Intensive structural analysis revealed that the isolate P2 was identical with cytotoxic and antiviral rhamnolipid B,[5,6] previously isolated from *P. aeruginosa*.



P2 (Rhamnolipid B)

Rhamnolipid B was first characterized by chemical degradation[5] and its structure containing two moles of each rhamnose and β-hydroxydecanoic acid was confirmed by stepwise enzymatic synthesis.[7] The connectivity within the disaccharide portion was first suggested to be a 1,3-linkage.[5] Later, rhamnose moeities were revised to be in 1,2-linkage by periodate oxidation and methylation.[8] However, NMR spectral data for the rhamnolipid have not yet been reported to show

Table 1. NMR Data for the Antifungal substance P2 (^{1}H at 500 MHz, ^{13}C at 125 MHz in CD_3OD)

Carbon No	^{13}C, δ	^{1}H, (m, *J* in Hz)	HMBC[a]
1	172.9		2, 3, 13
2	41.3	2.53 (dd, 15.1, 7.8)	3
		2.46 (dd, 15.1, 5.0)	
3	74.8	4.06-4.11 (m)	1′, 2
4	33.9	1.53-1.56 (m)	2
5	25.7	1.25-1.35 (m)	3, 4
6	30.8	1.25-1.35 (m)	4
7	30.3	1.25-1.35 (m)	
8	32.94[b]	1.25-1.35 (m)	10
9	23.7	1.25-1.35 (m)	10
10	14.4	0.89 (t, 7.1)	
11	178.2		12, 13
12	42.5	2.49 (dd, 15.1, 7.7)	13
		2.44 (dd, 15.1, 4.9)	
13	73.7	5.27-5.33 (m)	12
14	35.4	1.57-1.63 (m)	12
15	26.3	1.25-1.35 (m)	13, 14
16	30.5	1.25-1.35 (m)	14
17	30.3	1.25-1.35 (m)	
18	32.97[b]	1.25-1.35 (m)	20
19	23.7	1.25-1.35 (m)	20
20	14.4	0.89 (t, 7.3)	
1′	98.4	4.92 (d, 1.5)	3
2′	80.5	3.74 (dd, 3.2, 1.5)	1′, 1″
3′	71.9	3.79 (dd, 9.6, 3.2)	1′, 2′, 4′
4′	74.4	3.30 (t, 9.6)	2′, 3′
5′	70.1	3.65 (dq, 9.6, 6.2)	1′, 4′
6′	18.04[c]	1.23 (d, 6.2)	4′, 5′
1″	104.2	4.88 (d, 1.6)	2′
2″	72.0	3.96 (dd, 3.4, 1.6)	
3″	72.3	3.65 (dd, 9.5, 3.4)	1″, 2″, 4″
4″	73.9	3.38 (t, 9.5)	2″, 3″
5″	70.3	3.68 (dq, 9.5, 6.2)	1″, 4″
6″	18.07[c]	1.24 (d, 6.2)	4″, 4″

[a] Correlations to proton No. [b,c] Interchageable.

the 1,2-linkage between the disaccharide. Significance of the glycolipids as antifungal agents has been intensified with recent discovery of numerous related substances.[9~11]

We report herein the results of intensive spectral analysis of antifungal substance P2 (rhamnolipid B) and spectral evidence of 1,2-linkage by complete NMR signal assignments based on DEPT, H,H-COSY, HMQC, NOESY, and HMBC experiments.

Results and Discussion

A negative FAB mass spectrum indicated that the molecular weight of the purified antibiotic P2 was 650 Da, where the $[M-H]^-$ ion was observed at m/z 649. The molecular formula, $C_{32}H_{58}O_{13}$ was derived from detailed analysis of the ^{1}H and ^{13}C NMR spectral data (Table 1).

The ^{13}C NMR spectrum of P2 displayed signals for 32

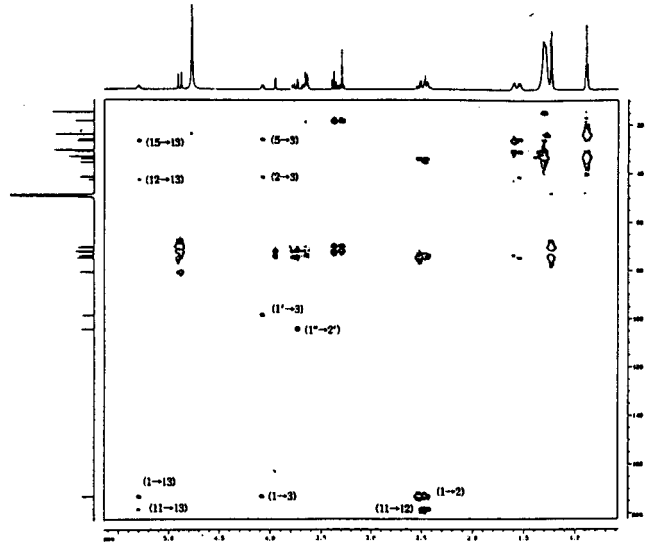


Figure 1. HMBC Spectrum of P2 in CD_3OD (C→H).

carbon atoms; ten of these occurred between δ 70 and δ 81 ppm, together with two signals at δ 98.41 and δ 104.18 ppm. These data suggested the presence of two carbohydrate moeities and two oxygenated carbon atoms. The DEPT experiments showed signals for four methyl, fourteen methylene, twelve methine, and two carbonyl carbons. Analysis of the ^{1}H NMR spectrum revealed the presence of two rhamnosyl residues, characterized by two doublet signals at δ 1.23 (J=6.2 Hz) and δ 1.24 (J=6.2 Hz) ppm that corresponded to two -CH_3 groups. The H,H-COSY and HMQC NMR spectra allowed the assignments of all the proton resonances of each rhamnosyl unit, and established the presence of the partial structures of two β-hydroxydecanoic acid moeities. The two doublets at δ 4.92 (J=1.5 Hz) and δ 4.88 (J=1.6 Hz) ppm were assigned to the anomeric H-1′ and H-1″ protons respectively. The two sets of doublet of doublets at δ 2.53 (dd, J=15.1, 7.8 Hz) and δ 2.46 (dd, J=15.1, 5.0 Hz) ppm corresponded to the α-protons (H-2) of one moeity of carboxylic acid and coupled to a H-3 proton in the range of δ 4.06-4.11 ppm. Another two sets of doublet of doublets at δ 2.49 (J=15.1, 7.7 Hz) and δ 2.44 (dd, J=15.1, 4.9 Hz) ppm were assigned to the α-protons (H-12) of the other acid moeity and coupled to a H-13 proton in the range of δ 5.27-5.33 ppm. Together with the HMBC experiments (Figure 1), all of the other signals were unambiguously assigned to provide the rhamnolipid structure.

Acid hydrolysis of the isolate P2 confirmed the presence of rhamnose and β-hydroxydecanoic acid. The lipophilic β-hydroxydecanoic acid was obtained by extraction of the acid hydrolyzate of P2 with Et_2O and methylated with diazomethane. The NMR, Mass, and TLC data of the resulting methyl ester was identical with those of an authentic racemic sample of methyl β-hydroxydecanoate which was prepared from the reaction of ethyl 3-oxodecanoate.[12] Concentration and recrystallization from MeOH/EtOAc gave rhamnose as a white solid. Chiral C-18 TLC (5 : 1 EtOAc/MeOH) analysis indicated that the rhamnose was identical with an authentic sample of α-L-rhamnose showing R_f value of 0.40 and 0.53 for two anomeric forms.

The connectivities between two rhamnosyl units and two β-hydroxydecanoic acid moeities were established by analysis of the HMBC and NOESY spectra. In the HMBC experiment, correlations were observed from H-13 to C-1 and C-11, H-3 to C-1 and C-1′. The most important HMBC correlations (Table 1) observed from H-2′ to C-1″ indicated that the connectivity between rhamnosyl groups were the 1,2-linkage. In addition, the NOESY spectrum confirmed the connectivities. NOEs were observed between the following pairs of protons: H-2′ and H-1″, H-1′ and H-3, H-1′ and H-4, H-3 and H-4, and H-13 and H-14. These experiments provided unequivocal evidence for the rhamnosyl linkage. It is notable that the downfield shift of the carbon signal for C-2′ by 8.5 ppm from that for C-2″ is further indication of the 1,2-linkage.

Experimental

Spectral Analysis. NMR spectra were recorded on a Bruker AMX 500 NMR spectrometer at 500 MHz for ^1H and 125 MHz for ^{13}C. ^1H and ^{13}C NMR chemical shifts are referenced to solvent peaks: δ_H 3.30 (residual CHD_2OD) and δ_C 49.0 for CD_3OD, and δ_H 7.26 (residual $CHCl_3$) and δ_C 77.0 for $CDCl_3$. Homonuclear ^1H connectivities were determined by the COSY experiment.[13] Heteronuclear ^1H-^{13}C connectivities were determined by HMQC[14] and HMBC experiments.[15] Mass spectra were recorded on a JEOL HX110A-HX110A tandem mass-mass spectrometer operating in the FAB or EP mode.

Antifungal Substance P2 (rhamnolipid B), 3-[3-[-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyloxy]-decanoyloxy]-decanoic acid. Isolation and purification of P2 from *P. aeruginosa.* strain B5 were previously reported.[3] Negative FABMS m/z (rel. int.) 649 $[M-H]^-$ (100), 479 (33), 183 (17); negative ESMS m/z (rel. int.) 649 $[M-H]^-$ (18), 479 (13), 205 (32), 169 (100), 163 (27); UV (MeOH) λ_{max} 219 nm; ^1H and ^{13}C NMR (CD_3OD), see Table 1.

Acid Hydrolysis of P2. The compound P2 (5 mg) was stirred in dioxane (2 mL) and 1 N HCl (2 mL) at 100 ℃ for 6 hr. The reaction mixture was partitioned between Et_2O and H_2O. The aqueous layer was concentrated and then recrystallized from MeOH/EtOAc to afford rhamnose as a white solid: TLC R_f 0.40 and 0.53 (5 : 1 EtOAc/MeOH, two anomeric forms, a Macherey-Nagel C-18 reverse-phase silica gel TLC chiralplate, visualization by anisaldehyde-sulfuric acid). An authentic sample of α-L-rhamnose (Sigma Chem. Co.) showed identical R_f value with the rhamnose. The Et_2O layer was washed with brine, concentrated, and methylated in Et_2O with diazomethane which was generated from the reaction of Diazald (Aldrich Chem. Co.) with KOH. The excess diazomethane was destroyed by adding acetic acid. The resulting reaction mixture was purified by chromatography (Merck silica gel 60, 70-230 mesh) with an elution gradient from 1 : 1 hexane/EtOAc to EtOAc to give methyl β-hydroxydecanoate as an oil, which was identical with an authentic compound in all aspects except optical properties.

Methyl β-hydroxydecanoate. Ethyl 3-oxodecanoate[12] (112 mg, 0.52 mmol) was dissolved in EtOH (3 mL) and followed by addition of $NaBH_4$ (40 mg, 1.06 mmol). The reaction mixture was partitioned between Et_2O and H_2O. The Et_2O layer was concentrated, dissolved in dioxane (2 mL) and H_2O (2 mL). After addition of Et_4NCl (60 mg, 0.41 mmol) and KOH (95 mg, 1.7 mmol), the mixture was stirred overnight. The reaction mixture was extracted with Et_2O to remove the unhydrolyzed ester. The resulting aqueous layer was acidified with concentrated HCl and extracted with Et_2O. The ethereal extract was concentrated and methylated with diazomethane in Et_2O. The resulting reaction mixture was chromatographed on silica gel (Merck silica gel 60, 70-230 mesh, 3 : 7 hexane/EtOAc) to give methyl β-hydroxydecanoate (85 mg) as an oil in 81% yield. ^1H NMR ($CDCl_3$) δ 0.82 (3H, t, J=7 Hz), 1.15-1.27 (m, 6H), 1.32-1.40 (m, 4H), 1.41-1.49 (2H, m), 2.35 (1H, dd, J=15, 8 Hz), 2.44 (1H, dd, J=15, 4 Hz), 2.68 (1H, bs), 3.65 (3H, s), 3.90-3.98 (1H, m) ppm; ^{13}C NMR ($CDCl_3$) δ 173.4, 68.0, 51.6, 41.1, 36.5, 31.7, 29.4, 29.1, 25.4, 22.6, 14.0 ppm.

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