New Surfactant Isolated from *Pseudomonas* 42A2

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***Pseudomonas* 42A2 is a gram negative rod isolated from a water sample. The new strain, when cultivated in mineral salt medium with olive oil as carbon source, produced a new surface active compound which was isolated and identified as dihydroxyoctadecenoic acid. No rhamnolipids were detected in the organic extract. The surface tension of the new compound is 30 mN/m at 50°C.**

Pseudomonas sp. is known by its capability to produce rhamnolipids (1-3) with surface active properties, when grown on nitrogen deficient medium with different carbon sources. When *Pseudomonas* 42A was incubated in mineral salt medium with olive oil as the sole carbon source, a substantial decrease in surface tension was detected. An active surface compound was isolated from the culture broth. The new compound was purified and identified as hydroxyoctanodecenoic acid. The new surfactant is slightly soluble in distilled water and has a surface tension of 30 mN/cm at 50°C.

EXPERIMENTAL PROCEDURES AND RESULTS

Organism and growth conditions. Pseudomonas 42A2 is a new strain isolated from contaminated waters as previously described (4). Its morphology and biochemical characteristics indicated it belongs to the genus *Pseudomonas*. The organism was grown on a mineral salt medium (4) with olive oil (2%) as the sole carbon source in a 500-ml baffled Erlenmeyer flask with 100 ml of medium at 30°C and 200 rpm.

Surface activity measurements. Surface tension was measured from the liquid culture and the cell-free broth centrifuged at 8000 × g for 20 min at 4°C with a Lauda Automatic Tensiometer TE-1 (Table 1). The critical micelle concentration, as an indirect measure of surfactant concentration, was determined by measuring the surface tensions of distilled water dilutions of the sample. Emulsification was measured by Vortexing equal volumes of the previously centrifuged culture medium with kerosene for two min and determining the percentage of volume occupied by the emulsion after 24 hr. All measurements were made at 20°C.

Chemical analysis. The new compound was isolated from the culture supernatant by acidification (pH 2) and extraction with ethyl acetate. Purification was carried out by column chromatography in Silicagel G 60 (Merck) using as solvent system $CHCl_3$:MeOH (9:1). A preliminary structure study was carried out with thin layer chromatography (TLC) reagents [Rf 0.73 with $CHCl_3$:MeOH:H2O (65:25:4)]. A structure with a polar lipid moiety by Molish reagent (brown spot) and a carboxyl group by bromocresol-green were assigned. The study with the Schiff reagent (5) gave negative results, indicating the two hydrosyl groups present [mass spectromety (MS) study] in the molecule were not vicinal. The infrared (IR) spectra showed the presence of an hydroxyl group (3600 cm^{-1}), an acid carbonyl group (3450 cm^{-1} and 1715 cm^{-1}) and a C-C isolated *trans* double bond (970 cm^{-1}).

Methylation and GLC study. Methylation was carried out by conventional methods (diazomethane). Ulterior gas liquid chromatography (GLC) study was made in a Hewlett-Packard 5890 instrument with a capillary column of cross-linked methyl silicone 12 m by 2 mm. The initial column temperature was 200 C, hold for 20 min; flow rate was 6 C/min to a maximum of 240 C, hold for 30 min. Flow rate of the carrier gas (helium) was one ml/min. Ignition and detector temperature were 250 and 260°C, respectively. The retention time of the new product was 7.75 min; oleic acid methyl esters and ricinoleic acid methyl esters presented a retention time of 3.94 min and 5.47 min, respectively. These retention times were in agreement with the presence of two hydroxyl groups. The ratio of retention volume and retention time of dihydroxy ester to monohydroxy ester is slightly larger than that of monohydroxy ester to nonhydroxy ester (6). GLC-MS results by CI with isobutane show the following signals at m/z: 367 M^+ + 39; 3%); 349 (310 + 39; 6%), 311 (M^+ $-H_2O$ +1; 100%), 292 (M^+ $-2H_2O$; 15%), 279 ($M^+-H_2O-OCH_3$; 6%).

We conclude the compound has a mol wt of 328, consistent with dihydroxyoctadecenoic acid methyl ester, C_{19} H_{36} O_4.

DISCUSSION

From the literature it is known that several *Pseudomonas* strains have been described to produced surface active compounds. The chemical characterization of these macromolecules resulted to be rhamnolipids (1-3) when cultivated either on hydrophobic or lipophylic carbon sources. We present for the first time a bacterial strain, named *Pseudomonas* 42A2 which produces a new extracellular surface active compound when incubated in mineral salt medium with olive oil as the sole carbon source. The active compound was extracted at pH 2

TABLE 1

Surface Active Properties of the Culture Broth and Culture Supernatant of *Pseudomonas 42A2* Measured at 20°C

	Surface tens (mN/cm)[a]			Interf.[b] tension	Emuls.
	ST	ST/4	ST/10	(mN/cm)	(%)
Culture broth	35	36	36	4.6	67.5
Cell free broth	33	33	38	4.8	61.5
Blank	44	48	49	12	0.0

[a]Surface tension of 1/4 and 1/10 dilutions of the sample with distilled water.

[b]Interfacial tension of the sample against kerosene.

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with ethyl acetate, purified by column chromatography and TLC and was shown to be dihydroxyoctadecenoic acid after IR and GLC-MS study. The new compound showed low solubility in water and its surface tension is 30 mN/m at 50°C at 100 ppm. These singular characteristics, the low water solubility in water and the high temperature activity of the new compound may permit it to find applications in the mining industry, in froth flotation of oxide ores (7) or in special markets either as a surfactant or as a chemical agent. The next step in our research is the study of the fermentation process of the new surfactant, the complete determination of the double bond and hydroxyl position in the C18 alkene chain by 13C NMR studies, electromagnetic (EM) and chemical degradation techniques, and the physicochemical behavior of the new surfactant.

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