appear not to be an essential part of the inhibition observed here. Phloretin is less than 1% as active against intestinal sugar transport as is the full glycoside.

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The synthesis of a rhamnolipid by enzyme preparations from *Pseudomonas aeruginosa*

Following the log phase of growth, *Pseudomonas aeruginosa* is known to produce and excrete a glycolipid into the media[1,2]. JARVIS AND JOHNSON[1] suggested the following structure for this glycolipid:

L-rhamnosyl-(1→3?)-L-rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoic acid

Recently a possible glycosyl donor for the synthesis of rhamnose glycosides, deoxyribosylthymine diphosphate rhamnose, has been isolated from several different types of bacteria[3,4]. The enzymic preparation of TDP-rhamnose has been described[5]. We wish to report the enzymic synthesis of the above rhamnolipid by enzymes occurring in *P. aeruginosa* (ATCC 7700) from TDP-rhamnose, β-hydroxydecanoyl-CoA, and either endogenous or exogenous acceptors.

Cell-free extracts of *P. aeruginosa* were prepared as previously described[5]. TDP-L-[^{14}C]rhamnose (23000 counts/min/μmole) was prepared enzymically[5]. Rhamnolipid was isolated and crystallized from cultures of *P. aeruginosa*. Acid hydrolysis of the rhamnolipid permitted the isolation of β-hydroxydecanoic acid (m.p., 44.5–45.0°,

Abbreviation: TDP, deoxyribosylthymine 5′-pyrophosphate.

uncorr.). The mixed anhydride method of WIELAND AND RUEFF[6] was employed to prepare β-hydroxydecanoyl-CoA. *P. aeruginosa* was grown in a medium containing sodium [1-^{14}C]acetate and the radioactive rhamnolipid was isolated and hydrolyzed to yield β-[^{14}C]hydroxydecanoic acid. Radioactivity was measured in a thin-window Geiger counter with a background of 1.5 counts/min (Nuclear Chicago Corporation).

Initially, it was observed that the incubation of TDP-[^{14}C]rhamnose and β-hydroxydecanoyl-CoA with sonic extracts of *P. aeruginosa* resulted in the formation of a radioactive material that could be isolated from the reaction mixture as an acid-insoluble, ether-soluble material. In experiments in which the reaction mixture was incomplete (no enzyme protein present) or was not incubated, the ether-soluble material failed to contain radioactivity. When the radioactive ether extract was chromatographed by an ascending technique on Schleicher and Schüll No. 589 red filter paper, using isobutyl ketone–glacial acetic acid–water (40:25:5) as the solvent, all of the radioactivity had an R_F of 0.6 ±0.05, identical to that of the rhamnolipid. In this solvent rhamnose has an R_F of 0.2 and sugar nucleotides remain at the origin. Rhamnolipid carrier was added to radioactive ether extracts and subsequently crystallized to constant specific activity (Table I). All of the radioactivity contained in the ether phase could be accounted for as crystallized rhamnolipid. Hydrolysis of the rhamnolipid yielded a single radioactive sugar as indicated by chromatography on Whatman No. 1 paper using a solvent system of *n*-butanol–pyridine–water (6:4:3). This sugar had the mobility of rhamnose and contained essentially all of the radioactivity present in the rhamnolipid crystals.

When β-[^{14}C]hydroxydecanoyl-CoA was incubated with the enzyme preparations in the presence of TDP-rhamnose, radioactive rhamnolipid could be isolated from the reaction mixture and crystallized to constant specific activity. No radioactive rhamnolipid was formed in the absence of TDP-rhamnose. In most enzyme preparations, small amounts of rhamnolipid were synthesized in the absence of added β-hydroxydecanoyl-CoA suggesting the presence of an endogenous acceptor. Rhamnolipid itself could not replace β-hydroxydecanoyl-CoA as acceptor of rhamnose.

However, acid hydrolysis of rhamnolipid under conditions which did not give complete cleavage of the glycolipid yielded products some of which could serve as an

TABLE I

RHAMNOLIPID CRYSTALLIZATION TO CONSTANT SPECIFIC ACTIVITY

The reaction mixture contained: 0.3 μmole of TDP-[^{14}C]rhamnose, 0.32 μmole of β-hydroxydecanoyl-CoA, 1.5 μmoles of glutathione, 0.35 ml of sonic extract of *Pseudomonas aeruginosa*, 15 μmoles of Tris, 5 μmoles of $MgCl_2$ in a total volume of 1.65 ml; pH 8.0. After incubation at 37° for 2 h, the sample was acidified and extracted with ether as described in Table II. Non-radioactive crystalline rhamnolipid carrier (22.5 mg) was added to the ether solution. The ether was removed under nitrogen and the rhamnolipid crystallized from the solvents indicated. An aliquot (about 1.5 mg) of each crop of crystals was plated in an area of 1.0 cm^2 and the radioactivity determined.

Crystallization	*Solvent*	*Specific activity (counts/min/mg)*
1	Acetone–water	53.4
2	Acetone–water	52.3
3	Acetone–water	52.6
4	Acetone–water	58.3
5	Diethyl ether–*n*-hexane	46.8
6	Diethyl ether–*n*-hexane	58.9

TABLE II

ACCEPTORS FOR RHAMNOSE IN THE SYNTHESIS OF RHAMNOLIPID

The reaction mixture contained: 30 μmoles of potassium phosphate (pH 7.5), 0.097 μmoles of TDP-[^{14}C]rhamnose and 0.25 ml of the enzyme preparation (calcium phosphate gel eluate) in a total volume of 0.6 ml. After incubation for 2 h at 37° the reaction was stopped by heating at 100° for 2 min. Rhamnolipid carrier (0.6 mg) was added. The reaction mixture was adjusted to pH 2.0 and extracted 3 times with 1 ml of diethyl ether. The combined ether extracts were washed with 2 ml of 0.001 *N* HCl. The radioactivity of the ether phase was determined.

Acceptor added	*Radioactivity in ether phase (counts/min)*
None	4.0
β-Hydroxydecanoyl-CoA (0.14 μmole)	42.0
β-Hydroxydecanoyl-β-hydroxydecanoic acid (0.04 μmole)	168.00

acceptor of rhamnose from TDP-rhamnose. One of these active products isolated by chromatography on silica gel has been tentatively identified as β-hydroxydecanoyl-β-hydrodecanoic acid. The enzyme protein that catalyzes the addition of rhamnose to this dimer has been separated from much of the other protein extracted from *P. aeruginosa* by protamine precipitation, ammonium sulfate precipitation, and absorption and elution from calcium phosphate gel. Typical data obtained with this enzyme preparation are presented in Table II. It may be seen that this enzyme preparation is dependent upon the addition of β-hydroxydecanoyl-β-hydroxydecanoic acid, but still retains some ability to synthesize rhamnolipid from β-hydroxydecanoyl-CoA and TDP-[^{14}C]rhamnose.

The work currently in progress in our laboratory is focused on the separation of the enzymes involved and sequence of addition in the total synthesis of this rhamnolipid.

Note added in proof: While this work was in progress BARBER AND NEUFELD[7] have reported that TDP-rhamnose acts as a glycosyl donor in the enzymic synthesis of rutin. (Received December 8th, 1961).

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