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Isolation of Polyisoprenyl Alcohols from *Streptococcus faecalis*

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C_{55}-isoprenyl alcohol and its derivatives have been isolated from *Streptococcus faecalis* and characterized. The relative amounts present as free alcohol, neutral lipid esters, and phosphate ester derivatives were determined. The chain lengths, mass spectra, and *cis* to *trans* ratio of double bonds are reported.

Polyisoprenyl alcohols and various phosphate ester derivatives have been isolated from a variety of bacteria (4, 5, 13). The phosphate esters are intermediates in a wide variety of reactions leading to the synthesis of extracellular polysaccharides (6, 7, 12, 14, 18). It has been suggested that dephosphorylation of isoprenyl phosphate and the adenosine triphosphate-dependent rephosphorylation may be a mechanism for regulating cell envelope biosynthesis (11). In the present work, the isoprenyl alcohol and its derivatives have been isolated from another organism, *S. faecalis*, in order to compare their chemistry to those already known, and further to determine the relative proportion of esterified and free alcohol.

MATERIALS AND METHODS

Purification of free and combined polyisoprenyl alcohol derivatives: preparation of the extract. A 358-g amount (wet weight) of late log-phase cells of *Streptococcus faecalis* ATCC 9790 (*S. faecium*), grown as previously described (8), were subjected to sonic treatment first with 2 liters of chloroform-methanol (2:1) and 200 ml of glass beads (5μm diameter) in a Branson sonifier. After filtering off the solvents with a sintered-glass funnel, the residue was subjected to sonic treatment a second time with 600 ml of 6 M pyridinium acetate (pH 4.2)-butanol (1:2), and again filtered. The solvents were removed in vacuo. The chloroform-methanol extract weighed 0.92 g, and the butanol-pyridinium acetate extract weighed 11.5 g.

Silica gel chromatography. The two extracts were individually separated into polar and neutral lipids by chromatography on silicic acid. Each was suspended in acetone and applied to a 1.8 by 17.5 cm column packed in acetone. The column was then eluted with 500 ml of acetone to remove the neutral lipid. Successive elutions with 1 liter each of chloroform-methanol (2:1) and methanol were pooled as the polar lipid fraction.

Separation of the neutral lipids. The neutral lipids from both extracts were pooled (241 mg) and separated on a column of alumina (Brockman II-III, 2.5 by 50 cm), developed successively with 350 ml each of (i) hexane, (ii) 2% diethyl ether in hexane, (iii) 6% diethyl ether in hexane, (iv) 15% diethyl ether in hexane, and (v) diethyl ether. Each fraction was taken to dryness in vacuo and resuspended in cyclohexane, and a portion was chromatographed on thin layers of silica gel G developed in 1% methanol in benzene. The fraction that eluted with solvent iv contained a single spot, detected with iodine vapor, with R_F corresponding to C_{55}-ficaprenol (10). The fraction that eluted with solvent v contained a spot corresponding to C_{55}-ficaprenol and several other components. The compound corresponding to C_{55}-ficaprenol was separated by preparative thin-layer chromatography and pooled with fraction iv for a total yield of 21 mg.

Fractions that eluted with solvents i and ii were saponified by refluxing in 4 ml of 15% KOH in ethanol-water (17:3) for 20 min and were extracted with ether. Chromatography of a portion of the ether extract revealed a spot corresponding to C_{55}-ficaprenol that was not present before saponification. This material was purified by preparative thin-layer chromatography as above (yield, about 0.15 mg) and might have been derived from a fatty acid ester of the isoprenyl alcohol.

Separation of the polar lipid. The polar lipids (chloroform-methanol and methanol eluates of the silicic acid column) from the two extracts were pooled and saponified by the method of Dawson (2). The organic layer was resuspended in chloroform-methanol (2:1), and the isoprenyl phosphate derivatives were then precipitated by the addition of 12 ml of absolute ethanol (10). The white precipitate was redissolved in 2 ml of *tert*-butyl alcohol, and then 4 ml of 6 N HCl was added. The mixture was heated for 15 min at 100 C and then extracted three times with diethyl ether. The ether extracts were pooled, dried in vacuo, and resuspended in 1 ml of cyclohexane. The material was subjected to preparative

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thin-layer chromatography on silica gel G in 1% methanol in benzene, and the components corresponding to C_{55}-ficaprenol and the rearrangement products obtained by acid-catalyzed beta-elimination of isoprenyl phosphates (i.e., tertiary alcohols and hydrocarbons) (10) were eluted. The total yield of material derived from isoprenyl phosphates was 4.6 mg (primary alcohol, 0.9 mg; the two tertiary alcohols, 0.7 and 1.6 mg; and the hydrocarbon, 1.4 mg).

RESULTS AND DISCUSSION

Characterization of the C_{55}-isoprenyl alcohols from S. faecalis: mass spectra. Mass spectrometry was performed on each isoprenyl alcohol fraction. The spectrum of the free alcohol fraction, obtained after treatment with acetic anhydride in pyridine, showed the molecular ion at M^+ = 808 *m/e* and the predominant acetolysis derivative at *m/e* 748. Fragmentation occurred in units of *m/e* 68 (Fig. 1). The primary alcohol, tertiary alcohols, and hydrocarbons derived by acid hydrolysis of the polar lipid fraction gave similar spectra, except that *m/e* 808 was absent in the latter two compounds. The spectrum of the ester fraction was unsatisfactory due to the small amount of material present. The fragmentation pattern indicated that it had isoprene character, but the chain length was not accurately determined.

Nuclear magnetic resonance (NMR) analysis. Sufficient material from the free alcohol fraction was available to permit NMR spectroscopy. This was performed under conditions similar to that described by Feeney and Hemming (3). From the spectrum (Fig. 2), it was possible to calculate a ratio of *cis*- to *trans*-internal double bonds of 8 to 2 (i.e., there were 9 *cis* and 3 *trans* methyl groups).

Gas chromatography. The chain lengths of the various fractions of isoprenyl alcohol derivatives were determined by gas chromatography by a slight modification of the procedure of Wellburn and Hemming (16). The n = 11 isoprenyl alcohol vastly predominated, although small amounts of materials with other chain lengths were seen (Fig. 3).

The major form of C_{55}-polyisoprenol in *S. faecalis* was the unesterified alcohol (Table 1). A substantial portion (about 15%) was found in acid-labile esters, presumably the phosphate ester derivatives. Only a small amount was present as esters in the neutral lipid fraction in contrast to the case in the mold *Aspergillus fumigatus* (9) or pig liver (P. H. W. Butterworth, 1964, cited in reference 9) where the fatty acid ester fraction predominates. Like the isoprenyl alcohols isolated from *Esche-*

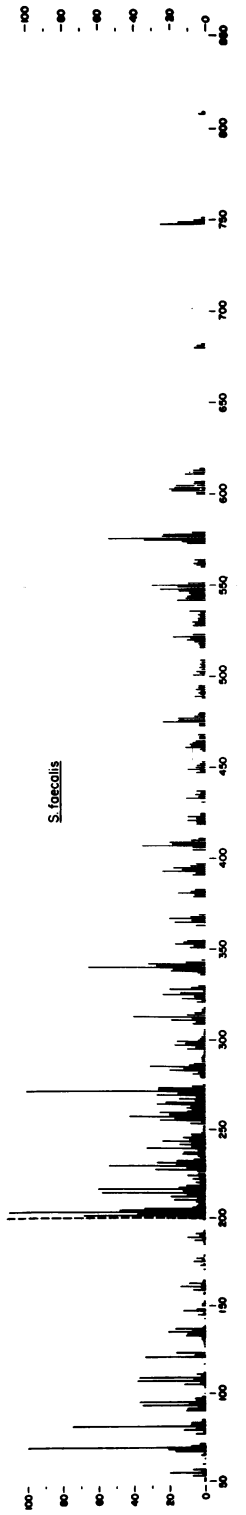

FIG. 1. *Mass spectrum of the isoprenyl alcohol isolated from S. faecalis. The free alcohol (1.1 mg) was acetylated with acetic anhydride (100 μliters) and pyridine (10 μliters). After drying in vacuo, a portion was subjected to mass spectrometry by direct probe injection into an LKB mass spectrometer. The ion current peaks were normalized to m/e 69 from m/e 50 to m/e 200 and to twenty times m/e 69 from m/e 200 to m/e 850.*

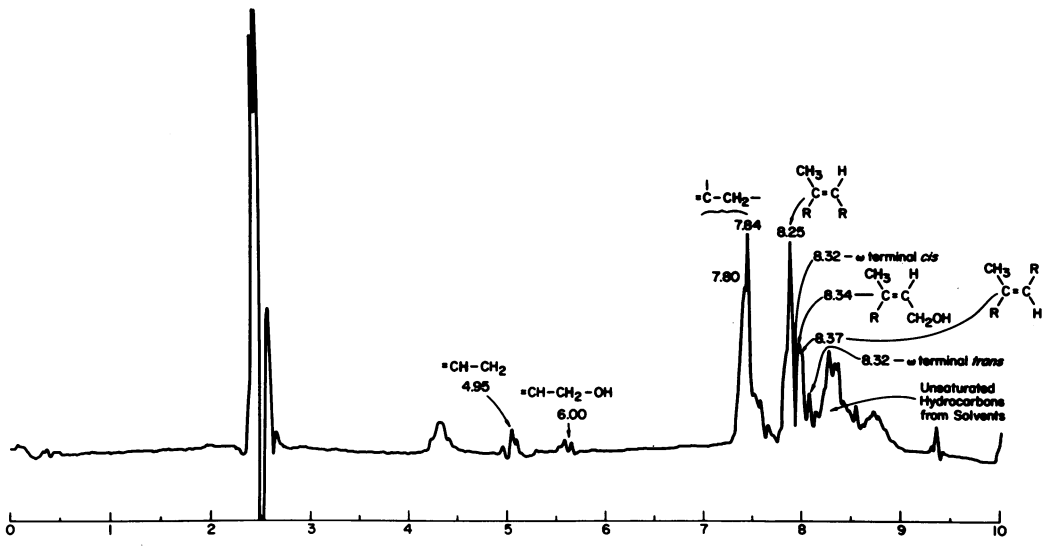

FIG. 2. *NMR spectrum of the polyisoprenyl alcohol isolated from S. faecalis. The NMR spectrum was obtained on a Varian 100 mHz by the Fourier transform (pulse) technique.*

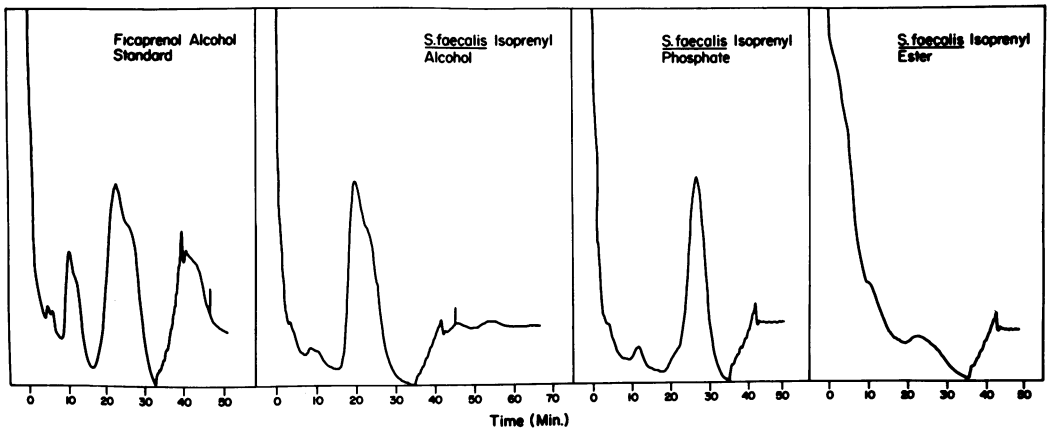

FIG. 3. *Gas-liquid chromatography of the isoprenyl derivatives isolated from S. faecalis. Chromatography was performed in glass columns (1 mm by 2 ft), packed with Chromosorb W, loaded with 1.5% SE-30 (Hewlett Packard, Medford, Mass.), and fitted with Covar seals and Swagelok fittings on the detector side of the column. A Packard series 9000 gas chromatograph with a flame ionization detector was used. The program ran isothermally for 35 min (sufficient time to allow the n = 11 to elute) at 289 C, and then the temperature was increased at 3 C/min to 320 C and held until n = 12 and any other peaks were eluted. The base line rose during programming due to increased column bleed, and this has been subtracted from the values in Table 1. The elution times were: n = 9, 6 min; n = 10, 10 min; n = 11, 22 min; and n = 12, 40 min.*

TABLE 1. *Levels and chain-length composition of isoprenyl derivatives from S. faecalis*

Fraction	Isoprenoid (mg/total lipid)	Ratio of polyisoprenols[a]			
		n = 9	*n* = 10	*n* = 11	*n* = 12
Isoprenyl alcohol	21.0	0.01	0.04	1	0.02
Isoprenyl esters	0.15	Trace	0.15	1	Not detected
Isoprenyl phosphate	4.62	Trace	0.05	1	Trace

[a] Ratio based on *n* = 11 as 1.

richia coli (15), *Micrococcus lysodeikticus* (6, 7), *Staphylococcus aureus* (5), *Lactobacillus plantarum* (4), *Lactobacillus casei* (13), and *Salmonella typhimurium* (18), the *S. faecalis* isoprenyl alcohols contain a predominance of the C_{55}-chain length. The NMR spectrum showed a ratio of internal *cis* to *trans* double bonds of 8 to 2, identical to that found in *S. aureus* (5), *L. plantarum* (4), and *M. lysodeikticus* (7), again demonstrating the close homology between isoprenyl derivatives from many organisms and their diverse functions.

The relatively high percentage of free alcohol among the isoprenyl alcohol derivatives isolated from *S. faecalis* (and possibly from related organisms) suggests that the free alcohols may have some role as a reserve pool whose availability for extracellular polysaccharide synthesis would then be controlled by phosphorylation and dephosphorylation (11, 17).

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