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Occurrence of Quinovosamine in Lipopolysaccharides of *Brucella* Species[1]

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Lipopolysaccharides obtained from *Brucella abortus*, *B. melitensis*, and *B. suis*, but not *B. canis*, were found to contain amino sugars identified as glucosamine and quinovosamine by cation exchange and thin-layer cellulose chromatography and ninhydrin degradation.

Miles and Pirie reported in 1939 the isolation of an antigenic endotoxin complex from *Brucella melitensis* which contained phospholipid, protein-like material and an *N*-formyl derivative of an aminopolyhydroxyl compound (14). Parnas et al. (16, 17), Lacave et al. (9, 10), and Renoux et al. (19) subsequently identified glucosamine in both virulent and avirulent strains of *Brucellae*. The present work reports the isolation and identification of both glucosamine and quinovosamine (2-amino-2,6-dideoxyglucose) in polysaccharides of *B. abortus*, *B. melitensis*, and *B. suis*, but not *B. canis*. Quinovosamine has been previously reported in *Achromobacter georgiopolitanum* (20), pneumococci (2), *Arizona*, some *Salmonella* species, *Proteus vulgaris* (12), and *Pseudomonas aeruginosa* (2, 5, 23, 26).

Lipopolysaccharides were prepared by extraction with aqueous ether (6) from *B. abortus* 19A, *B. suis* 3b, and *B. canis* RM-6-66, or by extraction with aqueous phenol from *B. abortus* 2308, *B. melitensis* 16M, and *B. suis* 1330 (4, 11). Degraded polysaccharides were prepared from the aqueous ether extracts by heating 1% lipopolysaccharide solutions in 1% acetic acid at 100 C for 90 min. The resulting polysaccharides were then *N*-acetylated, dialyzed, and lyophilized as previously described (18, 25). Free amino sugars were then obtained by hydrolysis of 10 mg of these polysaccharide samples per ml in 2 N HCl for 6 h at 100 C in sealed, evacuated tubes (25). HCl was removed by evaporation with repeated addition of absolute ethanol. Assay for concomitant ninhydrin reactive and reducing compounds by use of a modified amino acid analyzer (21) indicated the presence of glucosamine and quinovosamine in the amounts

TABLE 1. *Cation exchange chromatography of amino sugars*

Compound	$R_{glucosamine}$[a]		Micromoles milligram
	A	B	C
Glucosamine	1.00	1.00	
Galactosamine	1.11	ND	
Quinovosamine	1.28	1.41	
Fucosamine	1.43	ND	
B. abortus 19A			
Unknown 1	1.00	1.00	0.008
Unknown 2	1.27	1.38	0.20
B. abortus 2308			
Unknown 1	1.00	ND	0.016
Unknown 2	1.26	ND	0.050
B. suis 3b			
Unknown 1	1.00	1.00	0.018
Unknown 2	1.27	1.41	0.026
B. suis 1330			
Unknown 1	1.00	ND	ND
Unknown 2	1.27	ND	ND
B. melitensis 16M			
Unknown 1	1.00	ND	0.011
Unknown 2	1.29	ND	0.060
B. canis RM-6-66			
Unknown 1			
Unknown 2			

[a] Retention values relative to glucosamine. (A) Determined on a modified amino acid analyzer by the system of Steele et al. (21), eluted with 0.3 N sodium citrate buffer, pH 4.3; (B) Dowex 50-H^+ (8X, 200-400 mesh) column (1.5 by 100 cm), eluted with 0.33 N HCl (7). Amino sugars were located by reducing sugar assay (15) of neutralized samples ND, Not determined.

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TABLE 2. *Relative glucosamine mobility values of amino sugars chromatographed on thin-layer cellulose*[a]

Compound	Untreated solvents			Ninhydrin-degraded solvents		
	A	B	C	A	B	C
Glucosamine	1.00	1.00	1.00	1.49 ± 0.06	1.31 ± 0.01	3.00 ± 0.30
Quinovosamine	1.50 ± 0.04	1.78 ± 0.11	2.81 ± 0.22	2.25 ± 0.10	2.55 ± 0.07	5.18 ± 0.48
Arabinose				1.54 ± 0.02	1.31 ± 0.01	2.76 ± 0.20
B. suis 3b						
Unknown 1	0.98 ± 0.02	1.00 ± 0.02	1.00	1.45 ± 0.02	1.31	3.24 ± 0.06
Unknown 2	1.48 ± 0.04	1.84 ± 0.04	2.95 ± 0.06	2.17	2.60	5.58 ± 0.11
B. abortus 19A						
Unknown 1	1.00	1.00	1.00	1.55 ± 0.02	1.31 ± 0.01	2.76 ± 0.20
Unknown 2	1.52 ± 0.02	1.69 ± 0.02	2.68 ± 0.12	2.32 ± 0.07	2.49 ± 0.06	4.78 ± 0.08

[a] Although glucosamine retention values varied from one run to another, unknowns co-chromatographed with standards in each case. Values represent the arithmetic mean and range of variation of two or more chromatographic runs of both knowns and unknowns, relative to mobilities of glucosamine hydrochloride in the solvent systems indicated.

Solvent systems were composed as follows: (A) pyridine:ethyl acetate:acetic acid:water (5:5:1:3); (B) *N*-butanol:acetic acid:water (5:1:3); and (C) *N*-butanol:pyridine:water (6:4:3).

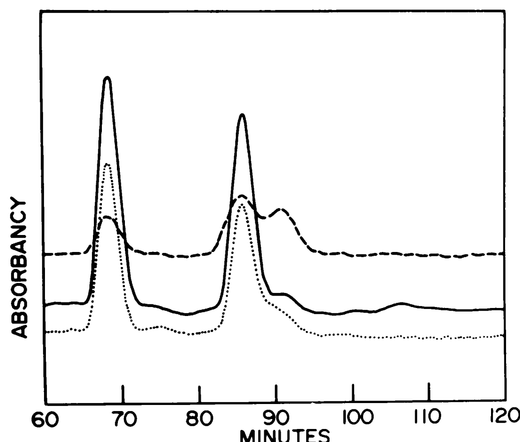

FIG. 1. *Amino sugar profile of a 22-h 2 N HCl hydrolysate of a mixture of 0.167 mg each of phenol-soluble lipopolysaccharides of Brucella abortus, B. melitensis, and B. suis. Cation exchange chromatography on amino sugar analyzer was done as described by Steele et al. (21). Symbols: ——, 570-nm ninhydrin trace; ····, 570-nm reducing group trace; -----, 440-nm ninhydrin trace. Glucosamine elution at 68 min, R_{GlcN} 1.0; quinovosamine at 86 min, (R_{GlcN} 1.27); and unknown at 92 min, (R_{GlcN} 1.35).*

shown in column C in Table 1. Glucosamine and quinovosamine appeared to be the major amino sugars detected (column A, Table 2). This identification was also supported by retention times relative to glucosamine obtained by preparative cation exchange chromatography of hydrolysates according to Gardell (7) (column B, Table 1). The isolated respective compounds co-chromatographed identically according to retention times of known standards (column A, Table 1). The isolated compounds gave strong 2-amino sugar reactions (3). Further confirmation of the identity of the unknowns as glucosamine and quinovosamine was obtained by thin-layer chromatography of the isolated compounds and their ninhydrin degradation products (22) (Table 2). Insufficient material was available to determine optical rotation.

Hydrolysates of the phenol-soluble lipopolysaccharide preparations corresponding to fraction V of Leong et al. (11) also yielded primarily glucosamine and quinovosamine (Fig. 1). In contrast to the report of Renoux et al. (19), only trace amounts of galactosamine were observed in the preparations we examined. However, as in the example shown in Fig. 1, small amounts of a third unidentified amino sugar-like compound, which elutes after quinovosamine, were observed in 22-h 2 N HCl hydrolysates of mixed samples of the three phenol-soluble preparations. It may be significant that the high 440/570 nm absorption ratio of the ninhydrin reaction product of this compound is similar to that of 4-amino-L-arabinose as reported by Volk et al. (24).

Two further observations deserve mention. First, approximately twice as much glucosamine and quinovosamine was observed in *B. abortus* polysaccharides prepared by aqueous phenol extraction as compared with those prepared by aqueous ether extraction. And finally, no amino sugars were found in the ultracentrifuge-sedimented (i.e., "purified") aqueous ether-extracted lipopolysaccharide preparations of *B. canis*. This suggests that *B. canis* could be a rough mutant which lacks the ability to produce a quinovosamine-containing side chain com-

mon to the other smooth-type *Brucellae*. This result and interpretation support and extend observations that the serologically similar antigens of smooth type *Brucellae* are not found in *B. canis* (4, 8, 13).

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