Isoprenoid Quinones in the Classification of Coryneform and Related Bacteria

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Menaquinones were the only isoprenoid quinones found in 85 of the 95 coryneform bacteria examined. Dihydromenaquinones having nine isoprene units were the main components isolated from *Corynebacterium bovis*, from other glutamic acid-producing strains, and from *Arthrobacter globiformis* and related species. Dihydromenaquinones with eight isoprene units were found in *Brevibacterium linens*, the remaining *Corynebacterium* species and strains probably belonging to the genus *Rhodococcus*. Tetrahydromenaquinones with eight isoprene units were found in *Arthrobacter simplex* and *Arthrobacter tumescens*, and with nine isoprene units in *Cellulomonas* and *Oerskovia*. *Kurthia* and *Curtobacterium* were characterized by menaquinones with seven and nine isoprene units, respectively, and *Microbacterium lacticum* and *Corynebacterium aquaticum* had comparable amounts of menaquinones with 10 and 11 isoprene units. Strains received as *Brevibacterium leucino-phagum*, *Corynebacterium autotrophicum*, *Corynebacterium nephridii*, *Mycobacterium flavum*, *Mycoplana rubra* and *Protaminobacter ruber* contained ubiquinones as their sole isoprenoid quinones. The isoprenoid quinone data correlate well with major trends in coryneform taxonomy and are of value in the classification of coryneform and related bacteria.

INTRODUCTION

In the current edition of *Bergey's Manual of Determinative Bacteriology* (Rogosa *et al.*, 1974), the coryneform group of bacteria accommodates the genera *Arthrobacter*, *Cellulomonas*, *Corynebacterium* and, tentatively, *Kurthia*, with *Brevibacterium* and *Microbacterium* described as genera *incertae sedis*. The genus *Curtobacterium* (Yamada & Komagata, 1972) proposed for certain 'motile brevibacteria' and the plant pathogens *Corynebacterium flaccumfaciens* and *Corynebacterium poinsettiae* should also be added to the coryneform group.

Improvements in the classification of coryneform and related bacteria have mainly been due to numerical phenetic studies (e.g. Bousfield, 1972; Jones, 1975), DNA base composition determinations (e.g. Bousfield, 1972; Skyring & Quadling, 1970; Yamada & Komagata, 1970*b*; Bowie *et al.*, 1972; Crombach, 1972; Starr *et al.*, 1975), wall analyses (e.g. Schleifer & Kandler, 1972; Keddie *et al.*, 1966; Yamada & Komagata, 1970*a*, 1972; Keddie & Cure, 1977) and mycolic acid analyses (Goodfellow *et al.*, 1976; Alshamaony *et al.*, 1976; Keddie & Cure, 1977). Preliminary data also suggest that isoprenoid quinone analyses may provide

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good characters for the classification of coryneform and related taxa (Yamada *et al.*, 1976; Collins *et al.*, 1977).

Isoprenoid quinones are widely distributed in bacteria and two major structural groups can be recognized, menaquinones (vitamins K) (I) and ubiquinones (coenzymes Q) (II). Menaquinones, 2-methyl-3-polyprenyl-1,4-naphthoquinones, display structural variations which may include the length and degree of hydrogenation of the polyprenyl side-chain, hydroxylation (Allen *et al.*, 1967) and possibly epoxidation (Friis *et al.*, 1967). Ubiquinones, 2,3-dimethoxy-5-methyl-6-polyprenyl-1,4-benzoquinones, also show variations in the length and degree of saturation of the side-chain (Thomson, 1971).

Coryneform and related bacteria can be divided into several groups on the basis of menaquinone composition (Yamada *et al.*, 1976; Collins *et al.*, 1977). Thus, while human and animal parasitic corynebacteria contain predominantly dihydromenaquinones with eight isoprene units, abbreviated as MK-8(H₂) (see *Biochemical Journal*, 1975, **147**, 15–21 for standard nomenclature and abbreviations), *Corynebacterium bovis*, *Corynebacterium glutamicum* and *Mycobacterium* strains have dihydromenaquinones with nine isoprene units, MK-9(H₂), as major components (Beau *et al.*, 1966; Yamada *et al.*, 1976; Collins *et al.*, 1977). Tetrahydromenaquinones with eight isoprene units have been found in *Nocardia* and *Arthrobacter simplex* strains (Yamada *et al.*, 1976; Collins *et al.*, 1977) whereas MK-9(H₂) has been reported in strains of *Cellulomonas* (Yamada *et al.*, 1976), *Oerskovia* (Collins *et al.*, 1977) and *Propionibacterium* (Schwartz, 1973; Sone, 1974).

This report presents the results of the mass spectral analysis of isoprenoid quinones from isolates bearing the labels *Arthrobacter*, *Brevibacterium*, *Cellulomonas*, *Corynebacterium*, *Curtobacterium*, *Kurthia*, *Microbacterium*, *Mycobacterium*, *Mycoplana*, *Nocardia*, *Oerskovia* and *Protaminobacter*.

METHODS

Cultures. The test strains (Table 1) were maintained on nutrient agar (Oxoid), and on Dorset Egg and Loeffler serum slopes (Cowan, 1974) at room temperature.

Cultivation. Strains were grown in shake flasks of nutrient broth [containing (per litre) 10 g peptone L34 (Oxoid), 8 g Lab Lemco L29 (Oxoid) and 5 g NaCl, pH 7.4] for 3 d at 30 °C. Cultures were checked for purity, killed with formaldehyde (1 %, v/v), harvested by centrifugation, washed with distilled water and freeze-dried.

Extraction and purification of isoprenoid quinones. Dried organisms were examined by the method of Collins *et al.* (1977).

Mass spectrometry. Mass spectra of the purified isoprenoid quinones were recorded on an AEI MS9 instrument using a direct insertion probe, an ionizing voltage of 70 eV and a temperature range of 190 to 220 °C.

Partial mass spectra of the isoprenoid quinones of all the strains listed in Table 1 have been deposited with the British Library Lending Division, Boston Spa, Yorkshire LS23 7BQ, as Supplementary Publication No. SUP 28009 (27 pages). Copies may be obtained from the BLLD on demand. Where possible, requests should be accompanied by prepaid coupons (held by many university and technical libraries and by the British Council).

Table 1. Designation and source of test strains

Taxon	No.	Strain designation/ source†	Taxon	No.	Strain designation/ source†
<i>Arthrobacter albidus</i>	1	*D6 (NCIB 10266)	<i>C. fascians</i>	1	C104 (NCPFB 1488)
<i>A. crystallopoietes</i>	1	C218 (NCIB 9499)	<i>C. flaccumfaciens</i>	1	C9 (NCPFB 559)
<i>A. globiformis</i>	1	*N549 (NCIB 8907)	<i>C. herculis</i>	1	*C85 (NCIB 9694)
<i>A. polychromogenes</i>	1	C219 (NCIB 10267)	<i>C. hoagii</i>	1	C24 (NCTC 10673)
<i>A. roseoparaffinus</i>	1	C112 (NCIB 10700)	<i>C. hydrocarboclastus</i>	2	*D8, D9 (K. Komagata, Ajinomoto Co., Kawa- saki, Japan; AJ 1386, AJ 1379)
<i>A. simplex</i>	2	*N295 (NCIB 8929); C272 (D. Jones, University of Leicester; C120)	<i>C. lilium</i>	1	C133 (NCIB 10337)
<i>A. tumescens</i>	1	C273 (NCIB 8914)	<i>C. manihot</i>	1	*C134 (NCIB 9097)
<i>A. ureafaciens</i>	1	C220 (NCIB 7811)	<i>C. melassecola</i>	1	C136 (NCIB 10336)
<i>A. variabilis</i>	1	D2 (NCIB 9455)	<i>C. minutissimum</i>	1	*D24 (NCTC 10288)
<i>Brevibacterium ammoniaenes</i>	1	C80 (NCIB 8143)	<i>C. murium</i>	1	C60 (NCTC 3655)
<i>B. divaricatum</i>	1	D3 (NCIB 9379)	<i>C. mycetoides</i>	1	C63 (NCTC 9864)
<i>B. fermentans</i>	1	*C122 (NCIB 9943)	<i>C. nebraskense</i>	2	H11, H12 (A. Vidaver, University of Nebraska, U.S.A.; Fur 1, Bennet Goth).
<i>B. flavum</i>	1	C81 (NCIB 9565)	<i>C. nephridii</i>	1	*C264 (DSM 20150)
<i>B. immariophilum</i>	1	C123 (NCIB 9544)	<i>C. poinsettiae</i>	1	C11 (NCPFB 844)
<i>B. lactofermentum</i>	1	C124 (NCIB 9567)	<i>C. pseudo- diphtheriticum</i>	1	C19 (NCTC 231)
<i>B. leucinophagum</i>	1	C125 (NCIB 9542)	<i>C. renale</i>	3	H1, H2, H4 (R. Yanagawa, Hokkaido University, Sapporo, Japan; 43, 45, 42)
<i>B. linens</i>	1	*C83 (NCIB 9909)	<i>C. rubrum</i>	1	C190 (NCIB 9433)
<i>B. paraffinolyticum</i>	1	C113 (NCIB 1160)	<i>C. xerosis</i>	1	C137 (NCIB 9956)
<i>B. roseum</i>	1	C82 (NCIB 9564)	<i>Curtobacterium citreum</i>	1	*C93 (NCIB 10702)
<i>B. saccharolyticum</i>	1	C127 (NCIB 9543)	<i>Curtobacterium luteum</i>	1	*C94 (NCIB 11029)
<i>B. stationis</i>	1	*C41 (ATCC 14403)	<i>Kurthia zopfii</i>	6	C37, C38, C72, C77 (D. Jones; C5, C6, C7, C205); *C45 (NCTC 404); C187 (NCIB 11555)
<i>B. stercorium</i>	1	C188 (NCIB 11161)	<i>Microbacterium ammoniaophilum</i>	1	C139 (NCIB 10335)
<i>B. sulfureum</i>	1	C79 (NCIB 10355)	<i>Microbacterium flavum</i>	1	*D4 (NCIB 8707)
<i>Cellulomonas biazotea</i>	1	*C221 (NCIB 8077)	<i>Microbacterium lacticum</i>	1	C91, C138 (NCIB 8541, 9919)
<i>Cell. cartalyticum</i>	1	C266 (DSM 20106)	<i>Mycobacterium flavum</i>	1	D5 (NCIB 10071)
<i>Cell. fimi</i>	1	C274 (NCTC 7547)	<i>Mycoplasma rubra</i>	1	C224 (NCIB 10409)
<i>Cell. flavigena</i>	1	*C111 (NCIB 8073)	<i>Nocardia cellulans</i>	1	N40 (NCIB 8868)
<i>Cell. subalbus</i>	1	C222 (NCIB 8075)	<i>Oerskovia xanthineolytica</i>	1	*C252 (NCIB 11025)
<i>Corynebacterium acetoacidophilum</i>	1	C129 (NCIB 9661)	<i>Protaminobacter ruber</i>	1	C251 (NCIB 2879)
<i>C. aquaticum</i>	1	*C84 (NCIB 9460)			
<i>C. autotrophicum</i>	5	C182 to C185 (H. C. Schlegel; 7C mut, 7CWA, 14g, 301b); C189 (NCIB 10809)			
<i>C. betae</i>	2	C3, C258 (NCPFB 363, 364)			
<i>C. bovis</i>	8	C95, C97 (NIRD 1689, 1928); C98 to C103 (J. E. Shreeve, Central Veterinary Laboratory, Weybridge, Surrey; DB223/75, DB132/75, DB210/75, DB30/75, DB94/75, 120B)			
<i>C. callunae</i>	1	C130 (NCIB 10338)			
<i>C. diphtheriae</i>	1	C13 (NCTC 3985)			
<i>C. equi</i>	8	C56 to C58 (NCTC 5649, 5650, 4219); D19 to D23 (H. R. Carne, Cambridge University; 20343, Jeffcott 1, 149, 1499, Jeffcott 11)			

* Type strain.

† ATCC, American Type Culture Collection; DSM, Deutsche Sammlung für Mikroorganismen; NCIB, National Collection of Industrial Bacteria; NCPFB, National Collection of Plant Pathogenic Bacteria; NCTC, National Collection of Type Cultures; NIRD, National Institute for Research in Dairying, Shinfield, Reading; C, D, H, N, laboratory numbers.

Table 2. Peaks corresponding to molecular ions in the mass spectra of menaquinones from organisms having dihydrogenated menaquinone main components with eight isoprene units

The main component in each series is denoted by + + +, any components greater than 50% of the main peak by + +, and all other significant components by +.

Menaquinone isoprenologue		MK-7					
Degree of hydrogenation <i>m/e</i>		(H ₂) 650	MK-8			MK-9	
			— 716	(H ₂) 718	(H ₄) 720	— 784	(H ₂) 786
<i>Arthrobacter roseoparaffinus</i>	c112	+	+	+++	—	—	—
<i>Brevibacterium linens</i>	c83	+	+	+++	—	—	—
<i>B. paraffinolyticum</i>	c113	+	+	+++	—	—	—
<i>B. stationis</i>	c41	+	+	+++	—	+	++
<i>B. sterolicum</i>	c188	+	+	+++	—	—	—
<i>Corynebacterium diphtheriae</i>	c13	+	+	+++	—	—	—
<i>C. equi</i>	c56	+	+	+++	—	—	+
	c57	+	+	+++	—	—	+
	c58	+	+	+++	—	—	—
	d19	+	+	+++	—	—	—
	d20	+	+	+++	—	—	—
	d21	+	+	+++	—	—	—
	d22	+	+	+++	—	—	—
	d23	+	+	+++	—	—	—
<i>C. fascians</i>	c104	+	+	+++	—	—	—
<i>C. hoagii</i>	c24	+	+	+++	—	—	—
<i>C. hydrocarboclastus</i>	d8	+	+	+++	—	—	—
	d9	+	+	+++	—	—	—
<i>C. minutissimum</i>	d24	+	+	+++	—	+	++
<i>C. murium</i>	c60	+	+	+++	—	—	—
<i>C. mycetoides</i>	c63*	+	+	+++	—	+	++
<i>C. pseudodiphtheriticum</i>	c19	+	+	+++	—	—	—
<i>C. renale</i>	h1	+	+	+++	+	—	—
	h2	+	+	+++	+	—	+
	h4	+	+	+++	+	—	—
<i>C. rubrum</i>	c190	+	+	+++	+	—	—
<i>C. xerosis</i>	c137	+	+	+++	—	—	+
<i>Microbacterium flavum</i>	d4	+	+	+++	—	—	+

* Significant amounts of MK-10(H₂) (*m/e* 854) were also detected.

RESULTS

Extracts of the test strains contained either menaquinones or ubiquinones which co-chromatographed with standards of vitamin K and ubiquinone-9, respectively. Menaquinones were detected in 85 strains and ubiquinones in the remaining 10. The most intense peaks in the mass spectra of menaquinones occurred at *m/e* 187 and 225 and were derived from the naphthoquinone nucleus. The ubiquinones produced base peaks at *m/e* 235, derived from the benzoquinone nucleus, and intense peaks at *m/e* 197. The mass spectra in the high mass region contained strong peaks corresponding to molecular ions (M⁺) with smaller peaks at M⁺ - 15 corresponding to the loss of a methyl group from the molecular ion. Details of the mass spectral analyses are shown in Tables 2 to 6.

Corynebacteria from animal sources, and strains labelled *Arthrobacter roseoparaffinus*, *Brevibacterium linens*, *Brevibacterium paraffinolyticum*, *Brevibacterium sterolicum*, *Corynebacterium fascians*, *Corynebacterium hydrocarboclastus*, *Corynebacterium rubrum* and *Microbacterium flavum* contained, as major components, menaquinones having eight isoprene units with one double-bond hydrogenated (Table 2). In contrast, the saprophytic corynebacteria, *Microbacterium ammoniaphilum* and variously labelled *Arthrobacter* and *Brevibacterium* strains possessed dihydromenaquinones with nine isoprene units as major components (Table 3). The single representatives of *Arthrobacter variabilis* and *Brevibacterium stationis* contained comparable amounts of MK-8(H₂) and MK-9(H₂) (Tables 2, 3).

Table 3. Peaks corresponding to molecular ions in the mass spectra of menaquinones from organisms having dihydrogenated menaquinone main components with nine isoprene units

The main component in each series is denoted by + + +, any components greater than 50 % of the main peak by + +, and all other significant components by +.

Menaquinone isoprenologue		MK-7	MK-8	MK-9	
Degree of hydrogenation <i>m/e</i>		(H ₂) 650	(H ₂) 718	— 784	(H ₂) 786
<i>Arthrobacter albidus</i>	D6	+	+	+	+++
<i>A. crystallopoietes</i>	c218*	+	+	+	+++
<i>A. globiformis</i>	N549*	+	+	+	+++
<i>A. polychromogenes</i>	c219	+	+	+	+++
<i>A. ureafaciens</i>	c220	+	+	+	+++
<i>A. variabilis</i>	D2	+	++	+	+++
<i>Brevibacterium ammoniagenes</i>	c80	+	+	+	+++
<i>B. divaricatum</i>	D3	+	+	+	+++
<i>B. flavum</i>	c81	+	+	+	+++
<i>B. immariophilum</i>	c123	+	+	+	+++
<i>B. lactofermentum</i>	c124	+	+	+	+++
<i>B. roseum</i>	c82	+	+	+	+++
<i>B. saccharolyticum</i>	c127	+	+	+	+++
<i>Corynebacterium</i>	c129	+	+	+	+++
<i>acetoacidophilum</i>					
<i>C. bovis</i>	c95	+	+	+	+++
	c97	+	+	+	+++
	c98	+	+	+	+++
	c99	+	+	+	+++
	c100	+	+	+	+++
	c101	+	+	+	+++
	c102	+	+	+	+++
	c103	+	+	+	+++
<i>C. callunae</i>	c130	+	+	+	+++
<i>C. herculis</i>	c85	+	+	+	+++
<i>C. lilium</i>	c133	+	+	+	+++
<i>C. melassecola</i>	c136	+	+	+	+++
<i>Microbacterium</i>	c139	+	+	+	+++
<i>ammoniophilum</i>					

* Significant amounts of MK-10(H₂) (*m/e* 854) were also detected.

Table 4. Peaks corresponding to molecular ions in the mass spectra of menaquinones from organisms having tetrahydrogenated menaquinone main components

The main component in each series is denoted by + + +, any components greater than 50 % of the main peak by + +, and all other significant components by +.

Menaquinone isoprenologue		MK-7	MK-8		MK-9	
Degree of hydrogenation <i>m/e</i>		(H ₄) 652	(H ₂) 718	(H ₄) 720	(H ₂) 786	(H ₄) 788
<i>Arthrobacter simplex</i>	N295	+	+	+++	—	—
	c272	+	+	+++	—	—
<i>A. tumescens</i>	c273	+	+	+++	—	—
<i>Brevibacterium fermentans</i>	c122	+	—	+	+	+++
<i>Cellulomonas biazotea</i>	c221	+	—	+	+	+++
<i>Cell. cartalyticum</i>	c266	+	—	++	+	+++
<i>Cell. fimi</i>	c274	+	—	+	+	+++
<i>Cell. flavigena</i>	c111	+	—	+	+	+++
<i>Cell. subalbus</i>	c222	+	—	+	+	+++
<i>Corynebacterium manihot</i>	c134	+	—	+	+	+++
<i>Nocardia cellulans</i>	N40	+	—	+	+	+++
<i>Oerskovia xanthineolytica</i>	c252	+	—	+	+	+++

Table 5. Peaks corresponding to molecular ions in the mass spectra of menaquinones from organisms having totally unsaturated menaquinone components

The main component in each series is denoted by + + +, any component greater than 50 % of the main peak by + +, and all other significant components by +.

Menaquinone isoprenologue		MK-6	MK-7 (H ₂)		MK-8	MK-9	MK-10	MK-11	MK-12
Degree of hydrogenation <i>m/e</i>		580	648	650	716	784	852	920	988
<i>Brevibacterium sulfureum</i>	c79	—	+	—	+	+++	+	—	—
<i>Corynebacterium aquaticum</i>	c84	—	—	—	+	+	++*	+++	+
<i>C. betae</i>	c3	—	+	—	+	+++	+	—	—
	c258	—	+	—	+	+++	—	—	—
<i>C. flaccumfaciens</i>	c9	—	+	—	+	+++	—	—	—
<i>C. nebraskense</i>	H11	—	+	—	+	+++	+	—	—
	H12	—	+	—	+	+++	+	—	—
<i>C. poinsettiae</i>	c11	—	+	—	+	+++	—	—	—
<i>Curtobacterium citreum</i>	c93	—	+	—	+	+++	—	—	—
<i>Curtobacterium luteum</i>	c94	—	+	—	+	+++	—	—	—
<i>Kurthia zopfii</i>	c37	+	+++	—	—	—	—	—	—
	c38	+	+++	—	—	—	—	—	—
	c45	+	+++	—	—	—	—	—	—
	c72	+	+++	—	—	—	—	—	—
	c77	+	+++	++	—	—	—	—	—
	c187	+	+++	—	—	—	—	—	—
<i>Microbacterium lacticum</i>	c91	—	—	—	+	+	++*	+++	+
	c138	—	—	—	+	+	++*	+++	+

* Intensity very similar to that of main peak.

Tetrahydrogenated menaquinones with eight isoprene units were found in *Arthrobacter simplex* and *Arthrobacter tumescens* (Table 4) whereas MK-9(H₄) was the predominant isoprenologue in *Brevibacterium fermentans*, *Corynebacterium manihot*, *Nocardia cellulans*, *Oerskovia xanthineolytica* and *Cellulomonas* strains (Table 4).

Kurthia zopfii strains contained MK-7 as the major isoprenologue with smaller quantities of MK-6 (Table 5). *Curtobacterium citreum* and *Curtobacterium luteum* possessed MK-9 as the major component, and essentially similar patterns were found in strains labelled *Brevibacterium sulfureum*, *Corynebacterium betae*, *Corynebacterium flaccumfaciens*, *Corynebacterium nebraskense* and *Corynebacterium poinsettiae* (Table 5). The mass spectra of the menaquinones from *Corynebacterium aquaticum* and *Microbacterium lacticum* are characteristic since they contain peaks of comparable intensity at *m/e* 852 and 920, corresponding to MK-10 and MK-11, respectively, and smaller peaks at *m/e* 716, 784 and 988 corresponding to the minor components MK-8, MK-9 and MK-12, respectively (Table 5).

The *Corynebacterium autotrophicum*, *Corynebacterium nephridii*, *Mycoplana rubra* and *Protaminobacter ruber* strains possessed ubiquinones as the sole isoprenoid quinones with Q-10 and Q-10(H₂) predominating (Table 6). The single strains of *Brevibacterium leucino-phagum* and *Mycobacterium flavum* contained Q-9 and Q-10, respectively, as the major isoprenologues.

Table 6. Peaks corresponding to molecular ions in the mass spectra of ubiquinones from organisms having ubiquinones as sole isoprenoid quinone components

The main component in each series is denoted by + + +, any components greater than 50 % of the main peak by + +, and all other significant components by +.

Ubiquinone isoprenologue		Q-8		Q-9		Q-10	
Degree of hydrogenation m/e		—	(H ₂)	—	(H ₂)	—	(H ₂)
		726	728	794	796	862	864
<i>Brevibacterium leucinophagum</i>	c125	+	—	+++	—	—	—
<i>Corynebacterium autotrophicum</i>	c182	—	—	+	+	++	+++
	c183	—	—	+	+	++	+++
	c184	—	—	+	+	++*	+++
	c185	—	—	+	+	++*	+++
	c189	—	—	+	+	++	+++
<i>C. nephridii</i>	c264	+	+	+	+	+++	++*
<i>Mycobacterium flavum</i>	D5	—	—	+	+	+++	+
<i>Mycoplana rubra</i>	c224	—	—	+	+	++*	+++
<i>Protaminobacter ruber</i>	c251	+	+	+	+	++*	+++

* Intensity very similar to that of main peak.

DISCUSSION

The strong chemical case for restricting the genus *Corynebacterium* to the human and animal strains and related saprophytic bacteria (Schleifer & Kandler, 1972; Goodfellow *et al.*, 1976; Keddle & Cure, 1977) is supported by data derived from the application of other taxonomic methods (Barksdale, 1970; Bousfield, 1972; Jones, 1975). Considering chemical characters only, *Corynebacterium sensu stricto* can be restricted to those bacteria having a wall chemotype IV, a DNA base composition in the range 48 to 59 mol % G+C and relatively low molecular weight mycolic acids (20 to 38 carbon atoms) (Keddle, 1976, 1978; Keddle & Cure, 1977, 1978; Minnikin *et al.*, 1978).

The true corynebacteria can be divided into two groups on the basis of menaquinone composition. Thus, the human and animal pathogens and related strains possess MK-8(H₂) as the major isoprenologue whereas *Corynebacterium bovis* and *Corynebacterium glutamicum* contain MK-9(H₂) (Collins *et al.*, 1977). The glutamic acid-producing strains *Arthrobacter albidus*, *Brevibacterium ammoniagenes*, *B. divaricatum*, *B. flavum*, *B. immariophilum*, *B. lactofermentum*, *B. roseum*, *B. saccharolyticum*, *Corynebacterium callunae*, *C. herculis* and *C. lilium* also contain MK-9(H₂) as the major component, have a wall type IV and, where tested, contain mycolic acids similar to those of true corynebacteria (Goodfellow *et al.*, 1976; Keddle & Cure, 1977). All of these strains should be transferred to the genus *Corynebacterium sensu stricto* (Barksdale, 1970; Minnikin *et al.*, 1978). *Corynebacterium melassecola* and *Microbacterium ammoniaphilum* also contain predominant amounts of MK-9(H₂) and so may belong to *Corynebacterium sensu stricto*, although further studies are required to confirm this. The menaquinone data do not contradict the view that many of the glutamic acid-producing nomenclatures should be reduced to synonymy with *C. glutamicum* (Abe *et al.*, 1967; Bousfield & Goodfellow, 1976).

The classification of strains labelled *Corynebacterium equi*, *C. fascians*, *C. hydrocarbonclastus* and *C. rubrum* in the genus *Rhodococcus* (Goodfellow & Alderson, 1977) is not contradicted by the menaquinone data. The type strain of *Brevibacterium linens* also possesses MK-8(H₂) as the major isoprenologue but, unlike the animal pathogenic corynebacteria and rhodococci, it does not contain mycolic acids (Goodfellow *et al.*, 1976). Strains labelled *Arthrobacter roseoparaffinus*, *Brevibacterium paraffinolyticum* and *B. sterolicum* contain major amounts of MK-8(H₂). Further studies are required to determine whether these taxa should be transferred to the genus *Corynebacterium* or to *Rhodococcus*.

The genus *Arthrobacter* is heterogeneous, and *Arthrobacter globiformis* and related strains can readily be distinguished from *A. simplex* and *A. tumescens* (Schleifer & Kandler, 1972; Keddle, 1974; Keddle & Cure, 1977). The menaquinone results underline this division since *A. globiformis*, *A. crystallopoietes*, *A. polychromogenes* and *A. ureafaciens* contain predominant amounts of MK-9(H₈) (Table 3) whereas *A. simplex* and *A. tumescens* possess major quantities of MK-8(H₄) (Table 4) as do nocardiae (Yamada *et al.*, 1976). However, unlike nocardiae, these arthrobacters do not contain MK-6(H₄) (Collins *et al.*, 1977). Our data cast doubt upon the report that *A. crystallopoietes* contains a ubiquinone (Kostiw *et al.*, 1972).

The integrity of the genus *Curtobacterium* is supported by the menaquinone data, for the curtobacteria, *Corynebacterium flaccumfaciens* and *Corynebacterium poinsettiae* contain MK-9 as the major isoprenologue. Further systematic work needs to be carried out on *Curtobacterium* (*Brevibacterium*) *testaceum* which has been reported to contain MK-11 (Yamada *et al.*, 1976). The *Brevibacterium sulfureum* and *Corynebacterium nebraskense* strains also contain menaquinones with nine isoprene units and should be compared further with curtobacteria. It has been suggested that *B. sulfureum* should be transferred to the genus *Arthrobacter* (Yamada & Komagata, 1972) and there is a good deal of evidence to support this (Yamada & Komagata, 1970*b*; Schleifer & Kandler, 1972; Keddle & Cure, 1977, 1978). However, the menaquinone data are at variance with the proposal to reclassify *B. sulfureum* in *Arthrobacter* and additional strains of this taxon need to be compared with arthrobacters and curtobacteria.

The present study (Table 4) confirms that MK-9(H₄) is the major isoprenologue of cellulomonads and oerskoviae (Collins *et al.*, 1977; Yamada *et al.*, 1976) but the walls of representatives examined contain ornithine and lysine, respectively (Prauser *et al.*, 1970; Keddle & Cure, 1977). *Brevibacterium fermentans*, *Corynebacterium manihot* and *Nocardia cellulans* also contain major amounts of MK-9(H₄) and all three have been found to contain lysine in their walls (Keddle & Cure, 1977; I. J. Bousfield, personal communication) suggesting a close affinity with *Oerskovia*.

Kurthia zopfii strains contain MK-7 as the predominant component and can thereby be distinguished from all coryneform and actinomycete strains examined to date (Collins *et al.*, 1977; Yamada *et al.*, 1976). Kurthiae can also be distinguished from the coryneforms by their morphology (Keddle & Rogosa, 1974) and low GC content (L. R. Hill, personal communication), and they have been recovered as a distinct homogeneous phenon in numerical phenetic surveys (Jones, 1975; Wilkinson & Jones, 1977).

The *Corynebacterium aquaticum* and *Microbacterium lacticum* strains also had an unusual menaquinone composition consisting of comparable amounts of MK-10 and MK-11 as the major isoprenologues. However, *C. aquaticum* can readily be distinguished from *M. lacticum*, because the former contains D-diaminobutyric acid as the major diamino acid of the peptidoglycan whereas the latter possesses L-lysine (Schleifer & Kandler, 1972).

The recovery of ubiquinones from *Brevibacterium leucinophagum*, *Corynebacterium autotrophicum*, *Corynebacterium nephridii*, *Mycobacterium flavum*, *Mycoplana rubra* and *Protaminobacter ruber* indicates that the classification of these taxa requires further study. Ubiquinones are common in Gram-negative bacteria (Thomson, 1971) but are rare in coryneform bacteria (Minnikin *et al.*, 1978). Jones & Weitzman (1974) found that *Brevibacterium leucinophagum* was Gram-negative and proposed its transfer to the genus *Acinetobacter*. The recovery of major amounts of ubiquinones with nine isoprene units from *B. leucinophagum* supports this assignment, as *Acinetobacter* strains also possess Q-9 as their major isoprenologue (Makula *et al.*, 1975).

The results of the present study confirm and extend earlier work (Yamada *et al.*, 1976; Collins *et al.*, 1977) which showed the value of isoprenoid quinone analyses in the classification of coryneform and related taxa, but further studies are necessary to evaluate the full potential of such analyses.

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