Distribution of Menaquinones in Actinomycetes and Corynebacteria

By M. D. COLLINS, T. PIROUZ AND M. GOODFELLOW

Department of Microbiology, The University, Newcastle upon Tyne NE1 7RU

AND D. E. MINNIKIN

*Department of Organic Chemistry, The University,
Newcastle upon Tyne NE1 7RU*

(Received 22 December 1976)

SUMMARY

Menaquinones were the only isoprenoid quinones found in 48 corynebacteria and actinomycete strains examined. Dihydromenaquinones having nine isoprene units were the main components isolated from *Gordona*, *Mycobacterium*, *Corynebacterium bovis*, *Corynebacterium glutamicum* and a strain labelled *Nocardia farcinica*, but dihydromenaquinones having eight isoprene units were characteristic of other *Corynebacterium* species and representatives of the 'rhodochrous' complex. Tetrahydromenaquinones having six and eight isoprene units were found in *Nocardia* strains and in a single strain of *Micropolyspora brevicatena*, which also contained mycolic acids similar in chain length to those of *Nocardia*. Menaquinones having nine isoprene units with from one to five double bonds hydrogenated were the main components in *Actinomadura madurae*, *Actinomadura pelletieri*, *Micropolyspora faeni*, *Oerskovia turbata* and *Streptomyces* strains. *Actinomadura dassonvillei* strains had a characteristic pattern of di-, tetra- and hexahydromenaquinones with 10 isoprene units which was slightly different from the pattern in mixtures of similar quinones from *Actinomyces israelii* and *Actinomyces viscosus*.

INTRODUCTION

The results of lipid analyses have clarified the classification of nocardioform and related bacteria (Lechevalier, 1976; Minnikin & Goodfellow, 1976). The most productive studies have involved analyses of mycolic acids (Lechevalier, Horan & Lechevalier, 1971; Maurice, Vacheron & Michel, 1971; Azuma *et al.*, 1974; Alshamaony, Goodfellow & Minnikin, 1976*a*; Alshamaony *et al.*, 1976*b*) and polar lipids (Khuller & Brennan, 1972; Pommier & Michel, 1973; Komura *et al.*, 1975).

Isoprenoid quinones are widely distributed in bacteria (Thomson, 1971) but systematic studies on their occurrence in actinomycetes and related taxa have not been carried out. The most common types of isoprenoid quinones are menaquinones (I) and ubiquinones (II). Menaquinones, 2-methyl-3-polyprenyl-1,4-naphthoquinones (I), show variations in the length and degree of unsaturation of the polyprenyl chain (Thomson, 1971), and in quinones isolated from *Streptococcus faecalis* (Baum & Dolin, 1965) and *Haemophilus parainfluenzae* (Lester, White & Smith, 1964) the methyl group in the 2-position is absent. Ubiquinones, 2,3-dimethoxy-5-methyl-6-polyprenyl-1,4-benzoquinones (II), also show variations in the polyprenyl chain, and in 'rhodoquinone', isolated from *Rhodospirillum rubrum*, the methoxyl group in the 3-position is replaced by an amino group (Thomson, 1971). Menaquinones and

ubiquinones are constituents of bacterial plasma membranes (Pennock, 1966) and possibly play a role in electron transport and oxidative phosphorylation (Brodie & Watanabe, 1966; Redfearn, 1966).

The isoprenoid quinone contents of only a few *Mycobacterium* and *Streptomyces* strains have been studied (Dunphy, Phillips & Brodie, 1971; Minnikin & Goodfellow, 1976). In *Mycobacterium* strains, the predominant menaquinones (I) had nine isoprenoid units with one double bond hydrogenated (dihydromenaquinones), abbreviated as MK-9(H₂) (see *Biochemical Journal*, 1975, **147**, 15–21 for standard nomenclature and abbreviations); but in a streptomycete strain, MK-9 occurred with MK-9(H₂, H₄, H₆ and H₈), MK-9(H₆) being the main component. Dihydromenaquinones are also found in corynebacteria; *Corynebacterium diphtheriae* and *C. rubrum* had MK-8(H₂) as the main component (Scholes & King, 1965; Beau, Azerad & Lederer, 1966) but in *C. createnovorans*, MK-9(H₂) predominated (Dunphy *et al.*, 1971).

Actinomycete and related strains representing well defined taxa have been studied to determine the value of isoprenoid quinone markers in their classification.

METHODS

Cultures. The test strains are listed in Table 1. The corynebacteria were maintained on Dorset Egg and Loeffler serum slopes (Cowan, 1974) and the remaining strains on yeast extract agar (Gordon & Mihm, 1962).

Cultivation. The corynebacteria and the *Oerskovia* strain were grown in shake flasks of nutrient broth (Oxoid) supplemented with 1% (v/v) Tween 80 for 2 to 3 days at 30 °C. The remaining strains were grown in shake culture for 3 to 10 days at 30 °C in modified Sauton's medium (Mordarska, Mordarski & Goodfellow, 1972); for the rhodochrous strains the latter was supplemented with vitamin B₁ (50 mg l⁻¹). Cultures were checked for purity at maximum growth, killed by shaking with formalin (1%, v/v), separated by centrifuging, washed with distilled water and freeze-dried. The *Actinomyces* strains were grown in BBL *Actinomyces* Broth in tightly stoppered 1 l bottles at 37 °C for 7 to 10 days.

Extraction and purification of menaquinones. Dried organisms (100 mg) were mixed with 20 ml chloroform/methanol (2:1, v/v) and the suspension was stirred continuously overnight. Organisms were then removed by filtration and the extract was evaporated to dryness under reduced pressure at low temperature (< 37 °C). Analytical thin-layer chromatography of quinones was performed using 0.5 mm layers of Merck Kiesel-gel HF₂₅₄ and a developing solvent consisting of petroleum ether (b.p. 60 to 80 °C)/diethyl ether (85:15, v/v). In this system menaquinones migrate much faster (*R_F* ~ 0.7) than ubiquinones (*R_F* ~ 0.4); vitamin K₁ (Sigma) and ubiquinone-50 (BDH) were used as chromatographic standards. Menaquinones were routinely detected on thin-layer chromatograms by brief irradiation with short-wave ultraviolet light (254 nm). Menaquinones were isolated from extracts by

Table 1. Designation and sources of test strains

Laboratory no.	Strain and source
A14	<i>Actinomadura dassonvillei</i> ; NCTC10488; ATCC23218. Type strain
A15	<i>Actinomadura dassonvillei</i> ; NCTC10489; ATCC23219
A114	<i>Actinomadura dassonvillei</i> ; laboratory strain
A16	<i>Actinomadura madurae</i> ; NCTC5654; ATCC19425. Type strain
A17	<i>Actinomadura madurae</i> ; NCTC1070
A19	<i>Actinomadura pelletieri</i> ; NCTC4162. Type strain
A40	<i>Actinomyces israelii</i> ; G. H. Bowden, Dept of Dental Bacteriology, The London Hospital Dental Institute, London E1; NCTC4860
A50	<i>Actinomyces viscosus</i> , G. H. Bowden; ATCC15987. Type strain
C200	<i>Actinomyces viscosus</i> ; T. Fillery, The London Hospital Dental Institute, 398A
C12	<i>Corynebacterium bovis</i> ; NCTC3224; ATCC7715. Type strain
C32	<i>Corynebacterium diphtheriae</i> ; NCTC3984; ATCC19409. Type strain
C7	<i>Corynebacterium equi</i> ; NCTC1621; ATCC6939. Type strain
C39	<i>Corynebacterium fascians</i> ; ATCC12974. Type strain
C35	<i>Corynebacterium flavidum</i> ; NCTC764; ATCC6940
D7	<i>Corynebacterium glutamicum</i> ; NCIB10025; ATCC13032
H5	<i>Corynebacterium pseudotuberculosis</i> ; P. Maximescu, Dr I. Cantacuzino Institute, Bucharest, Rumania, 992.
C17	<i>Corynebacterium renale</i> ; NCTC7448; ATCC19412. Type strain
C18	<i>Corynebacterium ulcerans</i> ; NCTC7910
C33	<i>Corynebacterium xerosis</i> ; NCTC9755
N654	<i>Gordona bronchialis</i> ; NCTC10667; ATCC25592. Type strain
N655	<i>Gordona rubra</i> ; NCTC10668; ATCC25593. Type strain
G14	<i>Micropolyspora brevicatena</i> ; S. T. Williams, Botany Dept, Liverpool University, E30; ATCC15725. Type strain
GI	<i>Micropolyspora faeni</i> ; J. Lacey, Rothamsted Experimental Station, Harpenden, A94; NCIB9984; ATCC15347. Type strain
M204	<i>Mycobacterium avium</i> ; H. B. Lee, Central Veterinary Laboratory, Weybridge, D4
M205	<i>Mycobacterium bovis</i> ; H. B. Lee, Otto strain
M206	<i>Mycobacterium intracellulare</i> ; H. B. Lee, strain MI
M207	<i>Mycobacterium johnei</i> ; H. B. Lee, strain 361F
M223	<i>Mycobacterium phlei</i> ; J. M. Grange, Cardiothoracic Institute, London SW3 6HP, 87; NCTC10008
M219	<i>Mycobacterium smegmatis</i> ; J. M. Grange, 607; ATCC607.
M209	<i>Mycobacterium tuberculosis</i> ; H. B. Lee, strain c
N317	<i>Nocardia asteroides</i> ; ATCC19247. Suggested working type (Sneath & Skerman, 1966)
N318	<i>Nocardia brasiliensis</i> ; ATCC19296. Type strain (Sneath & Skerman, 1966)
N36	<i>Nocardia caviae</i> ; NCTC1934; ATCC14629. Type strain
N34	<i>Nocardia farcinica</i> ; NCTC4524. Cotype (Sneath & Skerman, 1966)
N679	<i>Nocardia transvalensis</i> ; R. E. Gordon, Rutgers University, New Brunswick, U.S.A., 3426; NCTC2392
N681	<i>Nocardia vaccinii</i> ; R. E. Gordon, 3500; ATCC11092
C110	<i>Oerskovia turbata</i> ; NCIB10587. Type strain
A26	<i>Streptomyces somaliensis</i> ; M. Mariat, Institut Pasteur, Paris, 395
A29	<i>S. somaliensis</i> ; M. Mariat, 314
S6	<i>Streptomyces</i> sp. (neutrophilic strain); D. Dawson, Dept of Microbiology, University of Newcastle upon Tyne
S49	<i>Streptomyces</i> sp. (acidophilic strain); D. Dawson
'rhodochrous' complex	
Subgroup 14A*	
N324	'rhodochrous' strain (<i>Nocardia pellegrino</i>); ATCC15998
N325	'rhodochrous' strain (<i>N. pellegrino</i>); G. Castelnuovo, Istituto Superiore Di Sanita, Rome, 906B
Subgroup 14B*	
N31	'rhodochrous' strain (' <i>Mycobacterium rhodochrous</i> '); R. E. Gordon, w21
Subgroup 14C*	
N54	'rhodochrous' strain (' <i>Mycobacterium rhodochrous</i> '); ATCC13808; R. E. Gordon, 372 (<i>Rhodococcus rhodochrous</i>)
N61	'rhodochrous' strain (' <i>Mycobacterium rhodochrous</i> '); R. E. Gordon, w3408; ATCC4273
Subgroup 14D*	
N11	'rhodochrous' strain (<i>Nocardia erythropolis</i>); NCIB9158; ATCC4277
N53	'rhodochrous' strain (<i>Jensenia canicruria</i>); NCIB8147; ATCC11048

* Phena recovered by Goodfellow (1971).

preparative thin-layer chromatography; chloroform was used to elute the quinone from the silica gel.

Analysis of menaquinones. Ultraviolet spectra of menaquinones were recorded in hexane solution. Mass spectra of menaquinones were recorded on an AEI MS9 instrument using a direct insertion probe, an ionizing voltage of 70 eV and a temperature range of 200 to 230 °C.

Analysis of mycolic acids. Whole organisms (100 mg) of the *Micropolyspora* strains were degraded by acid methanolysis as described previously (Minnikin, Alshamaony & Goodfellow, 1975). Methyl esters of mycolic acids were detected and purified using the chromatographic system described above. Conditions for the analysis of mycolic esters by mass spectroscopy were the same as for the menaquinone samples.

Partial mass spectra of the menaquinones from all the strains (Table 1) have been deposited with the British Lending Library, Boston Spa, Yorkshire LS23 7BQ, as Supplementary Publication No. SUP 28006 (13 pages). Copies may be obtained from the BLL on demand. Where possible, requests should be accompanied by prepaid coupons (held by many university and technical libraries and by the British Council).

RESULTS

Components that co-chromatographed with vitamin K₁ were the only isoprenoid quinones detected by thin-layer chromatography in extracts of the strains studied (Table 1). After isolation by preparative thin-layer chromatography, representative samples were examined by ultraviolet spectroscopy and found to have absorption maxima at 242, 248, 260, 270 and 325 nm in accordance with published data for menaquinones (I) (Dunphy & Brodie, 1971).

The mass spectra of the menaquinone samples showed, as expected from published data (Azerad & Cyrot-Pelletier, 1973), intense peaks at m/e 187 and 225 derived from the naphthoquinone nucleus and strong peaks corresponding to molecular ions (M^+). All mass spectra contained small peaks corresponding to loss of a methyl group from molecular ions ($M^+ - 15$) and occasionally peaks of low intensity were seen at $M^+ + 16$. The latter may be due to partial oxidation of the sample during its preparation for mass spectrometry; epoxides have been detected as minor components, possibly artefacts, in ubiquinone samples (Friis, Daves & Folkers, 1967). These minor peaks do not, however, interfere with the analysis of menaquinones by mass spectrometry provided they are not mistaken for molecular ions. The results of the mass spectral analyses of the menaquinones isolated from the test strains are shown in Tables 2 to 5.

The predominant menaquinone found in the majority of the *Corynebacterium* strains and representatives of the 'rhodochrous' complex (Goodfellow, 1971) was MK-8(H₂) (Table 2). However, *Corynebacterium bovis* and *C. glutamicum* strains had MK-9(H₂) as the main menaquinone component, as did representatives of *Gordona*, *Mycobacterium* and *Nocardia farcinica* N34 (Table 3).

The mass spectra of the menaquinones from other *Nocardia* strains and *Micropolyspora brevicatena* G14 (Table 4) contained predominant peaks at m/e 720 corresponding to tetrahydromenaquinones MK-8(H₄). Small peaks were found for MK-7 components at m/e 652 but larger peaks attributable to MK-6(H₄) were seen at m/e 584. Small unexplained peaks were observed at m/e 596, 12 mass units higher than the major peaks at m/e 584. Thin-layer chromatographic analysis of a whole-organism methanolysate of *Micro. brevicatena* G14 revealed a component migrating in a similar manner to methyl esters of mycolic acids isolated from *Nocardia* (Minnikin *et al.*, 1975). Mass spectroscopic analysis of this component gave a series of peaks from m/e 682 to 814 attributable to anhydromycolic esters

Table 2. Peaks corresponding to molecular ions in the mass spectra of menaquinones isolated from strains of *Corynebacterium* and the 'rhodochrous' complex

The main component of 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	— 716	(H ₂) 718	(H ₄) 720	(H ₂) 786
<i>C. equi</i> C7	+	+	+++	+	+
<i>C. flavidum</i> C35	+	—	+++	++	+
<i>C. diphtheriae</i> C32	+	—	+++	—	—
<i>C. fascians</i> C39	+	+	+++	—	—
<i>C. pseudotuberculosis</i> H5	+	+	+++	—	—
<i>C. renale</i> C17	+	+	+++	—	—
<i>C. ulcerans</i> C18	+	+	+++	—	—
<i>C. xerosis</i> C33	+	+	+++	—	—
'rhodochrous' complex	14A* N324	+	+++	—	—
	N325	+	+++	—	—
	14B* N31	+	+++	—	—
	14C* N54	+	+++	+	—
	N61	+	+++	—	—
	14D* N11	+	+++	—	—
	N53	—	+++	—	—

* Subgroups of the 'rhodochrous' complex (Goodfellow, 1971).

Table 3. Peaks corresponding to molecular ions in the mass spectra of menaquinones isolated from strains of *Corynebacterium*, *Gordona*, *Mycobacterium* and *Nocardia farcinica*

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-8			MK-9			MK-10
Degree of hydrogenation <i>m/e</i>	— 716	(H ₂) 718	(H ₄) 720	— 784	(H ₂) 786	(H ₄) 788	(H ₂) 854
<i>C. bovis</i> C12	+	+	—	+	+++	—	+
<i>C. glutamicum</i> D7	—	+	—	+	+++	—	—
<i>G. bronchialis</i> N654	—	+	—	+	+++	—	—
<i>G. rubra</i> N655	—	+	—	+	+++	—	—
<i>Myco. avium</i> M204	—	+	—	+	+++	+	—
<i>Myco. bovis</i> M205	+	+	—	+	+++	—	—
<i>Myco. intracellulare</i> M206	—	+	—	+	+++	—	—
<i>Myco. phlei</i> M223	—	+	+	+	+++	+	—
<i>Myco. johnei</i> M207	—	+	—	+	+++	—	—
<i>Myco. smegmatis</i> M219	—	+	—	+	+++	—	—
<i>Myco. tuberculosis</i> M209	—	+	—	+	+++	—	—
<i>N. farcinica</i> N34	—	+	+	+	+++	+	—

corresponding to mycolic acids having from 46 to 56 carbon atoms (Alshamaony *et al.*, 1976a). In contrast, the *Micro. faeni* strain did not contain mycolic acids.

A variety of menaquinone patterns were found in actinomycete strains which lack mycolic acids (Table 5). The streptomycetes had MK-9(H₆) as the main component but MK-9(H₄) and MK-9(H₈) occurred in comparable proportions. Related menaquinone patterns (Table 5) were found for representatives of *Micro. faeni*, *Actinomadura madurae* and *Actinomadura pelletieri* but the proportions of MK-9 components were different. In *Actinomyces*

Table 4. *Peaks corresponding to molecular ions in the mass spectra of menaquinones isolated from strains of Nocardia and Micropolyspora brevicatena*

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-6	MK-7	MK-8		
	(H ₄) 584	(H ₄) 652	(H ₂) 718	(H ₄) 720	(H ₆) 722
<i>N. asteroides</i> N317	++	+	+	+++	—
<i>N. brasiliensis</i> N318	++	+	+	+++	—
<i>N. caviae</i> N36	++	+	+	+++	—
<i>N. transvalensis</i> N679	++	+	+	+++	—
<i>N. vaccinii</i> N681	++	—	+	+++	+
<i>Micro. brevicatena</i> G14	+	+	+	+++	—

israelii and *Actinomyces viscosus* strains, MK-10(H₄) predominated but in representatives of *Actinomadura dassonvillei* comparable amounts of MK-10(H₂) and MK-10(H₆) were also present. *Oerskovia turbata* C110 had MK-9(H₄) as the major component.

DISCUSSION

Numerical phenetic surveys indicate that *Actinomadura*, *Actinomyces*, *Corynebacterium sensu stricto* (Goodfellow, Collins & Minnikin, 1976), *Mycobacterium*, *Nocardia sensu stricto* (Cross & Goodfellow, 1973), *Oerskovia* and the 'rhodochrous' complex form distinct taxa of equivalent rank (Goodfellow, 1971; Holmberg & Nord, 1975; Bousfield & Goodfellow, 1976). Since the distribution of character frequencies is strongly U-shaped for homogeneous groups (Sneath, 1976), chemical markers can be expected to be of value in classification of these taxa. The present preliminary studies suggest that menaquinone analyses can provide good characters for the classification of actinomycete and related taxa.

The menaquinones of organisms with a wall chemotype IV (Lechevalier, 1976) which also contain mycolic acids fell into three distinct patterns (Tables 2, 3 and 4). In particular, menaquinone analyses distinguished *Nocardia sensu stricto* and *Micro. brevicatena* which contained a mixture of tetrahydromenaquinones with six and eight isoprene units from *Corynebacterium*, *Gordona*, *Mycobacterium* and the 'rhodochrous' complex. Further evidence for a close relationship between *Nocardia* and *Micro. brevicatena* was indicated by the mycolic acids of the latter which were similar (46 to 56 carbon atoms) to those found in *Nocardia sensu stricto* (Alshamaony *et al.*, 1976a; Minnikin & Goodfellow, 1976). In contrast, *Micro. faeni* G1 did not contain mycolic acids and had a menaquinone composition similar to streptomycetes and some actinomadurae. These chemical data therefore suggest that the genus *Micropolyspora* is heterogeneous and is in need of further study.

The recovery of dihydromenaquinones with nine isoprene units from mycobacteria was consistent with earlier reports (Dunphy *et al.*, 1971) and the presence of similar menaquinones in *Nocardia farcinica* N34 was not unexpected as it has other mycobacterial properties (Lechevalier, 1976). Magnusson (1976) has proposed that *N. farcinica* N34, and similar strains, be reclassified as *Mycobacterium farcinogenes* var. *senegalense* Chamoiseau 1973. Our data cast doubt on the classification of a strain labelled *Mycobacterium flavum* which was reported to contain a ubiquinone (Erickson, 1971).

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				MK-10				MK-11			
	(H ₄) 652	(H ₆) 654	(H ₈) 656	(H ₁₀) 658	(H ₄) 720	(H ₆) 722	(H ₈) 724	(H ₁₀) 726	(H ₄) 784	(H ₆) 786	(H ₈) 788	(H ₁₀) 790	(H ₄) 852	(H ₆) 854	(H ₈) 856	(H ₁₀) 858	(H ₄) 922	(H ₆) 924		
Degree of hydrogenation <i>ml/e</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
<i>Actinomyces israelii</i> A40	—	—	—	—	—	—	—	—	+	+	+	—	—	+	+	—	—	—		
<i>Actinomyces viscosus</i> A50	—	—	—	—	—	—	—	—	+	+	—	—	—	+	+	—	—	—		
<i>Actinomadura dassonvillei</i> A14	—	—	—	—	—	—	—	—	+	+	—	—	+	+	+	—	—	—		
A15	—	—	—	—	—	—	—	—	+	+	—	—	+	+	+	—	—	—		
A114	—	—	—	—	—	—	—	—	+	+	+	—	+	+	+	—	—	—		
<i>Actinomadura madurae</i> A16	—	—	—	—	—	—	—	—	+	+	+	+	—	—	—	—	—	—		
A17	—	—	—	—	—	—	—	—	+	+	+	+	—	—	—	—	—	—		
<i>Actinomadura pelletieri</i> A19	—	—	—	—	—	—	—	—	+	+	+	+	—	—	—	—	—	—		
<i>Streptomyces somaliensis</i> A26	+	+	+	—	—	—	—	—	+	+	+	+	—	—	—	—	—	—		
A29	+	+	+	—	—	—	—	—	+	+	+	+	—	—	—	—	—	—		
<i>Streptomyces</i> sp. s6	—	—	—	—	—	—	—	—	+	+	+	+	—	—	—	—	—	—		
s49	—	—	—	—	—	—	—	—	+	+	+	+	—	—	—	—	—	—		
<i>Micropolyspora faeni</i> G1	—	—	—	—	—	—	—	—	+	+	+	+	—	—	—	—	—	—		
<i>Oerskovia turbata</i> C110	—	—	—	—	—	—	—	—	+	+	+	+	—	—	—	—	—	—		

Menaquinone composition also distinguished the animal corynebacteria and representatives of the 'rhodochrous' complex (Goodfellow, 1971), which contained a dihydromenaquinone with eight isoprene units, from mycobacteria and gordonae. *Corynebacterium fascians* C39 should not be classified with the corynebacteria but in the 'rhodochrous' complex (Goodfellow *et al.*, 1976). The significance of the occurrence of major amounts of MK-9(H₂) in *C. bovis* and *C. glutamicum* must await comparative studies on more strains though a similar result has been recorded for a strain labelled *Corynebacterium creatovorans* (Dunphy *et al.*, 1971). It may also be of interest that a strain labelled *Brevibacterium thio genitalis* (Okazaki, Kanzaki & Fukuda, 1968), which contained mycolic acid and produced glutamic acid, had MK-9(H₂) as the major menaquinone component (Kanzaki *et al.*, 1974).

The relationship between the 'rhodochrous' complex and the genus *Gordona* is a complex one (Bousfield & Goodfellow, 1976; Tsukamura, 1975) and it has recently been proposed that they be merged to form the resurrected genus *Rhodococcus* containing 10 species (Goodfellow & Alderson, 1977). Our preliminary findings suggest that menaquinone analyses will provide good characters for the classification of rhodococci.

The menaquinone composition of streptomycetes is in accord with previous studies (Dunphy *et al.*, 1971) and closely related patterns of MK-9 components were also found in *Actinomadura madurae*, *Actinomadura pelletieri* and *Micro. faeni* strains. The different pattern of partially hydrogenated MK-10 components found in *Actinomadura dassonvillei* strains supports the previous distinction of this species from the other two *Actinomadura* taxa on the basis of spore morphology (Williams *et al.*, 1976), and content of madurose (Lechevalier & Gerber, 1970), fatty acid (Agre, Efimova & Guzeva, 1975) and pigment (Lechevalier, Lechevalier & Gerber, 1971). *Actinomyces israelii* and *Actinomyces viscosus* had a distinct pattern with MK-10(H₄) as the predominant component.

Cross & Goodfellow (1973) did not allocate *Oerskovia* to a family. In a subsequent numerical taxonomic study, Jones (1975) found that the genus *Oerskovia* shared a higher affinity with coryneform bacteria than with actinomycetes. It was, therefore, interesting that *Oerskovia turbata* C110 had MK-9(H₄) as the major component, a quinone previously found to be characteristic of the genus *Propionibacterium* (Schwartz, 1973; Sone, 1974).

These preliminary analyses suggest that isoprenoid quinones may prove to be of value in the classification of actinomycetes and corynebacteria. However, a greater number of strains need to be studied and the present results, based entirely on mass and ultraviolet spectroscopy, should be substantiated by further detailed studies.

The authors are indebted to Drs G. H. Bowden and J. M. Hardie for the gift of freeze-dried *Actinomyces*; to Mr H. B. Lee, Central Veterinary Laboratory, Weybridge, for freeze-dried mycobacteria; to colleagues who kindly provided cultures (Table 1); to Ms G. Alderson and Mr A. Temple for maintaining cultures and preparing cells and to P. Kelly for mass spectroscopy. M.D.C. gratefully acknowledges receipt of a Luccock Scholarship (Medical Scholarships and Research Committee, Faculty of Medicine, Newcastle upon Tyne), and M.G. and D.E.M. wish to thank the Medical Research Council for support (grant G974/522/S).

REFERENCES

- AGRE, N. S., EFIMOVA, T. P. & GUZEVA, L. N. (1975). Heterogeneity of the genus *Actinomadura* Lechevalier and Lechevalier. *Mikrobiologiya* **44**, 252–257.
- ALSHAMAONY, L., GOODFELLOW, M. & MINNIKIN, D. E. (1976*a*). Free mycolic acids as criteria in the classification of *Nocardia* and the 'rhodochrous' complex. *Journal of General Microbiology* **92**, 188–199.
- ALSHAMAONY, L., GOODFELLOW, M., MINNIKIN, D. E. & MORDARSKA, H. (1976*b*). Free mycolic acids as criteria in the classification of *Gordona* and the 'rhodochrous' complex. *Journal of General Microbiology* **92**, 183–187.
- AZERAD, R. & CYROT-PELLETIER, M. O. (1973). Structure and configuration of the polyprenoid side chain of dihydromenaquinones from myco- and corynebacteria. *Biochimie* **55**, 591–603.
- AZUMA, I., OHUCHIDA, A., TANIYAMA, T., YAMAMURA, Y., SHOJI, K., HORI, M., TANAKA, Y. & RIBI, E. (1974). The mycolic acids of *Mycobacterium rhodochrous* and *Nocardia corallina*. *Biken's Journal* **17**, 1–19.
- BAUM, R. H. & DOLIN, M. I. (1965). Isolation of 2-solaneyl-1,4-naphthoquinone from *Streptococcus faecalis*, 10 C1. *Journal of Biological Chemistry* **240**, 3425–3433.
- BEAU, S., AZERAD, R. & LEDERER, E. (1966). Isolément et caractérisation des dihydromenaquinones des myco- et corynébactéries. *Bulletin de la Société de chimie biologique* **48**, 569–581.
- BOUSFIELD, I. J. & GOODFELLOW, M. (1976). The 'rhodochrous' complex and its relationships with allied taxa. In *The Biology of the Nocardiae*, pp. 39–65. Edited by M. Goodfellow, G. H. Brownell and J. A. Serrano. London: Academic Press.
- BRODIE, A. F. & WATANABE, T. (1966). Mode of action of vitamin K in micro-organisms. *Vitamins and Hormones* **24**, 447–463.
- CHAMOISEAU, G. (1973). *Mycobacterium farcinogenes*, agent causal du farcin du boeuf en Afrique. *Annales de Microbiologie* **124A**, 215–222.
- COWAN, S. T. (1974). *Manual for the Identification of Medical Bacteria*, 2nd edn. Cambridge: Cambridge University Press.
- CROSS, T. & GOODFELLOW, M. (1973). Taxonomy and classification of the actinomycetes. In *Actinomycetales: Characteristics and Practical Importance*, pp. 11–112. Edited by G. Sykes and F. A. Skinner. London: Academic Press.
- DUNPHY, P. J. & BRODIE, A. F. (1971). The structure and function of quinones in respiratory metabolism. *Methods in Enzymology* **XVIII**, 407–461.
- DUNPHY, P. J., PHILLIPS, P. G. & BRODIE, A. F. (1971). Separation and identification of menaquinones from microorganisms. *Journal of Lipid Research* **12**, 442–449.
- ERICKSON, S. K. (1971). The respiratory system of the aerobic, nitrogen-fixing, Gram-positive bacterium, *Mycobacterium flavum* 301. *Biochimica et biophysica acta* **245**, 63–69.
- FRIIS, P., DAVES, G. D. & FOLKERS, K. (1967). New epoxyubiquinones. *Biochemistry* **6**, 3618–3624.
- GOODFELLOW, M. (1971). Numerical taxonomy of some nocardioform bacteria. *Journal of General Microbiology* **69**, 33–80.
- GOODFELLOW, M. & ALDERSON, G. (1977). The actinomycete-genus *Rhodococcus*: a home for the 'rhodochrous' complex. *Journal of General Microbiology* **100**, 99–122.
- GOODFELLOW, M., COLLINS, M. D. & MINNIKIN, D. E. (1976). Thin-layer chromatographic analysis of mycolic acid and other long-chain components in whole-organism methanolysates of coryneform and related taxa. *Journal of General Microbiology* **96**, 351–358.
- GORDON, R. E. & MIHM, J. H. (1962). Identification of *Nocardia caviae* (Erikson) nov. comb. *Annals of the New York Academy of Sciences* **98**, 628–636.
- HOLMBERG, K. & NORD, C.-E. (1975). Numerical taxonomy and laboratory identification of *Actinomyces* and *Arachnia* and some related bacteria. *Journal of General Microbiology* **91**, 17–44.
- JONES, D. (1975). A numerical taxonomic study of coryneform and related bacteria. *Journal of General Microbiology* **87**, 52–96.
- KANZAKI, T., SUGIYAMA, Y., KITANO, K., ASHIDA, Y. & IMADA, I. (1974). Quinones of *Brevibacterium*. *Biochimica et biophysica acta* **348**, 162–165.
- KHULLER, G. K. & BRENNAN, P. J. (1972). The polar lipids of some species of *Nocardia*. *Journal of General Microbiology* **73**, 409–412.
- KOMURA, I., YAMADA, K., OTSUKA, S. & KOMAGATA, K. (1975). Taxonomic significance of phospholipids in coryneform and nocardioform bacteria. *Journal of General and Applied Microbiology* **21**, 251–261.
- LECHEVALIER, M. P. (1976). The taxonomy of the genus *Nocardia*: some light at the end of the tunnel. In *The Biology of the Nocardiae*, pp. 1–38. Edited by M. Goodfellow, G. H. Brownell and J. A. Serrano. London: Academic Press.
- LECHEVALIER, M. P. & GERBER, N. N. (1970). The identity of madurose with 3-O-methyl-D-galactose. *Carbohydrate Research* **13**, 451–454.
- LECHEVALIER, M. P., HORAN, A. C. & LECHEVALIER, H. A. (1971). Lipid composition in the classification of nocardiae and mycobacteria. *Journal of Bacteriology* **105**, 313–318.

- LECHEVALIER, H. A., LECHEVALIER, M. P. & GERBER, N. N. (1971). Chemical composition as a criterion in the classification of actinomycetes. *Advances in Applied Microbiology* **14**, 47-72.
- LESTER, R. L., WHITE, D. C. & SMITH, S. L. (1964). The 2-desmethyl vitamin K₂'s. A new group of naphthoquinones isolated from *Haemophilus parainfluenzae*. *Biochemistry* **3**, 949-954.
- MAGNUSSON, M. (1976). Sensitin tests as an aid in the taxonomy of *Nocardia* and its pathogenicity. In *The Biology of the Nocardiae*, pp. 236-265. Edited by M. Goodfellow, G. H. Brownell and J. A. Serrano. London: Academic Press.
- MAURICE, M. T., VACHERON, M. T. & MICHEL, G. (1971). Isolément d'acides nocardiques de plusieurs espèces de *Nocardia*. *Chemistry and Physics of Lipids* **7**, 9-18.
- MINNIKIN, D. E. & GOODFELLOW, M. (1976). Lipid composition in the classification and identification of nocardiae and related taxa. In *The Biology of the Nocardiae*, pp. 160-219. Edited by M. Goodfellow, G. H. Brownell and J. A. Serrano. London: Academic Press.
- MINNIKIN, D. E., ALSHAMAONY, L. & GOODFELLOW, M. (1975). Differentiation of *Mycobacterium*, *Nocardia* and related taxa by thin-layer chromatographic analysis of whole-organism methanolysates. *Journal of General Microbiology* **88**, 200-204.
- MORDARSKA, H., MORDARSKI, M. & GOODFELLOW, M. (1972). Chemotaxonomic characters and classification of some nocardioform bacteria. *Journal of General Microbiology* **71**, 77-86.
- OKAZAKI, H., KANZAKI, T. & FUKUDA, H. (1968). L-Glutamic acid fermentation. Part V. Behaviour of oleic acid in an oleic acid-requiring mutant. *Agriculture and Biological Chemistry* **32**, 1464-1470.
- PENNOCK, J. F. (1966). Occurrence of vitamins K and related quinones. *Vitamins and Hormones* **24**, 307-329.
- POMMIER, M. T. & MICHEL, G. (1973). Phospholipid and acid composition of *Nocardia* and nocardoid bacteria as criteria of classification. *Biochemical Systematics* **1**, 3-12.
- REDFEARN, E. R. (1966). Mode of action of ubiquinones (coenzymes Q) in electron transport systems. *Vitamins and Hormones* **24**, 465-488.
- SCHOLES, P. B. & KING, H. K. (1965). Isolation of a naphthaquinone with partly hydrogenated side chain from *Corynebacterium diphtheriae*. *Biochemical Journal* **97**, 766-768.
- SCHWARTZ, A. C. (1973). Terpenoid quinones of the anaerobic *Propionibacterium shermanii*. *Archiv für Mikrobiologie* **91**, 273-279.
- SNEATH, P. H. A. (1976). An evaluation of numerical taxonomic techniques in the taxonomy of *Nocardia* and allied taxa. In *The Biology of the Nocardiae*, pp. 74-101. Edited by M. Goodfellow, G. H. Brownell and J. A. Serrano. London: Academic Press.
- SNEATH, P. H. A. & SKERMAN, V. B. D. (1966). A list of type and reference strains of bacteria. *International Journal of Systematic Bacteriology* **16**, 1-133.
- SONE, N. (1974). Isolation of a novel menaquinone with a partly hydrogenated side chain from *Propionibacterium arabinosum*. *Journal of Biochemistry* **76**, 133-136.
- THOMSON, R. H. (1971). Naturally occurring quinones. London: Academic Press.
- TSUKAMURA, M. (1975). Numerical analysis of the relationship between *Mycobacterium*, rhodochrous group and *Nocardia* by use of hypothetical median organisms. *International Journal of Systematic Bacteriology* **25**, 329-335.
- WILLIAMS, S. T., SHARPLES, G. P., SERRANO, J. A., SERRANO, A. A. & LACEY, J. (1976). The micromorphology and fine structure of nocardioform organisms. In *The Biology of the Nocardiae*, pp. 102-140. Edited by M. Goodfellow, G. H. Brownell and J. A. Serrano. London: Academic Press.