Macquarimicins, Microbial Metabolites from *Micromonospora*

II. Isolation and Structural Elucidation

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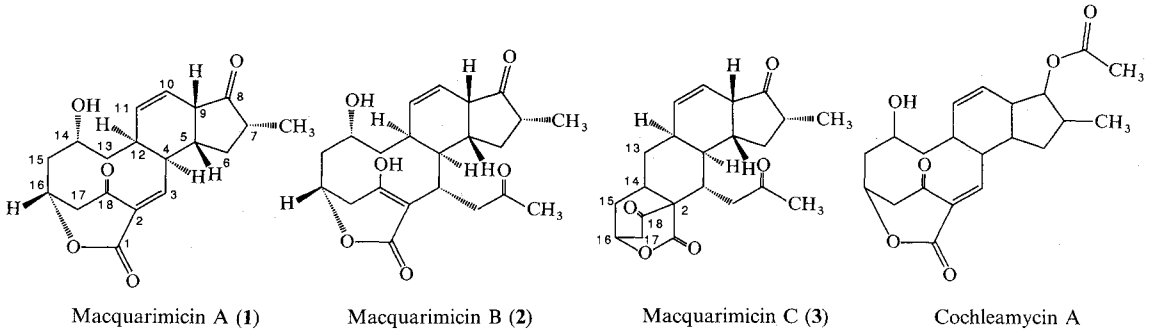
A novel series of carbocyclic compounds has been isolated from two related *Micromonospora* cultures. The C-19 and C-22 macquarimicins represent different end products on a similar biosynthetic scheme. 1-D and 2-D homonuclear and heteronuclear NMR experiments allowed assignemnt of the basic structures of the macquarimicins. An X-ray structure of macquarimicin B suggested the stereochemistry for the series which was not discernible from spectroscopic data alone.

In preliminary communications we have reported[1,2] on the isolation and structure of a novel antibiotic, macquarimicin A (**1**), from two strains of *Micromonospora chalcea*. More recently, the related cochleamycins A and B have been reported[3] from a *Streptomyces* species. This paper describes, in detail, the isolation and structural elucidation of macquarimicin A and two related metabolites, macquarimicins B (**2**) and C (**3**), from *Micromonospora chalcea*. A companion paper[4] describes the production and biological activity of the macquarimicins. The structure of macquarimicin B was confirmed and the stereochemistry defined by single crystal X-ray diffraction. The X-ray structure of macquarimicin B also served as a presumptive model for the stereochemistry of macquarimicins A and C.

Isolation of the Macquarimicins

Upon harvest of 17 liters of whole broth of culture AB 969J-62, the mycelial mass and solids were removed by centrifugation and filtration. The culture filtrate was then adjusted to pH 7 and treated with XAD-4 resin (2 liters). A methanol eluate (4 liters) of this resin was concentrated to a crude oil which was subjected to size exclusion column chromatography on a Sephadex LH-20 column developed in methanol. Active fractions from this column were combined, concentrated to dryness and subjected to countercurrent chromatography on an Ito multi-layered coil planet centrifuge in a solvent system of chloroform - carbon tetrachloride - methanol - water (1 : 1 : 1 : 1) with the lower phase stationary. Active fractions from this chromatography were combined and concentrated to yield 80 mg of pure macquarimicin A.

Macquarimicin B was isolated from a separate harvest of this same culture *via* a different isolation scheme; Upon harvest, 30 liters of whole broth was treated with XAD-4 (4 liters), which was subsequently washed with methanol (8 liters). The methanol concentrate was partitioned between EtOAc - EtOH - H_2O (1.5 : 0.5 : 1.0 liters) and the lower phase was concentrated to an oil. This oil was partitioned between *n*-PrOH - *n*-BuOH - H_2O (0.5 : 1.0 : 1.5 liters) and the upper phase from this partition was concentrated to an oil. This oil was subjected to countercurrent chromatography on an Ito multi-layered coil planet centrifuge in a solvent system



Macquarimicin A (**1**) Macquarimicin B (**2**) Macquarimicin C (**3**) Cochleamycin A

Table 1. Physico-chemical properties of the macquarimicins.

	Macquarimicin A	Macquarimicin B	Macquarimicin C
Mass spec	*m/z* 330	*m/z* 388	*m/z* 370
Formula	$C_{19}H_{22}O_5$	$C_{22}H_{28}O_6$	$C_{22}H_{26}O_5$
Infrared	3550, 3490, 1735, 1705, 1618 cm^{-1}	3525, 3130, 1725, 1688, 1658, 1628 cm^{-1}	2868, 2859, 2824, 1756, 1725, 1668, 1602 cm^{-1}
Ultraviolet	Neutral, acidic: 224 nm (ε 800) Basic: 274 nm (ε 1,600)	Neutral, acidic, basic: 205 nm (ε 10,800), 255 (9,100), 278 (8,700)	Neutral, basic: 206 nm (ε 1,100), 286 (340), 382 (420) Acidic: 206 nm (ε 1,200), 286 (310), 376 (500)

of EtOAc - EtOH - H_2O (3 : 1 : 2) with the lower phase stationary. Active fractions from this chromatography were combined and concentrated to yield 4 mg of pure macquarimicin B (**2**).

Macquarimicin C was isolated from a separate, 30 liter harvest of this culture treated with SP-207 (3 liters) and eluted with methanol (8 liters). The methanol concentrate was partitioned between EtOAc - EtOH - H_2O (1.5 : 0.5 : 1.0 liters) and the lower phase concentrated to an oil. This oil was chromatographed over Sephadex LH-20 developed in MeOH - $CHCl_3$ (1 : 1). Active fractions from this column were combined and concentrated to an oil which was subjected to countercurrent chromatography on a droplet instrument in a solvent system of hexane - EtOAc - MeOH - H_2O (1 : 2 : 1 : 2) in the descending mode. Active fractions from this chromatography were combined and concentrated to an oil which was then subjected to three successive countercurrent chromatographies on an Ito multi-layered coil planet centrifuge in solvent systems; hexane - CH_3CN - MeOH - H_2O (5 : 5 : 4 : 2) lower phase stationary, hexane - EtOAc - MeOH - H_2O (1 : 1 : 1 : 1), upper phase stationary and hexane - EtOAc - MeOH - H_2O (8 : 2 : 5 : 5), lower phase stationary, respectively to yield 2.1 mg of pure macquarimicin C (**3**).

Table 2. ^1H NMR and ^{13}C NMR assignments for macquarimicin A (in $CDCl_3$).

Carbon No.	Carbon shift	Proton shift
1	165.7 (Q)	
2	136.4 (Q)	
3	155.2 (CH)	6.78 (d, 1H, J=11.1 Hz)
4	38.6 (CH)	3.44 (ddd, 1H, J=11.1, 6.1, 2.4 Hz)
5	34.7 (CH)	2.64 (mult, 1H)
6	33.1 (CH_2)	2.24 (ddd, 1H, J=12.0, 7.0, 1.1 Hz), 1.48 (q, 1H, J=12.0 Hz)
7	43.9 (CH)	2.31 (dqd, 1H, J=12.0, 6.6, 1.1 Hz)
7-CH_3	13.9 (CH_3)	1.11 (d, 3H, J=6.6 Hz)
8	217.3 (Q)	
9	45.7 (CH)	3.11 (obscured, 1H)
10	123.5 (CH)	5.65 (dt, 1H, J=10.2, 3.0 Hz)
11	127.7 (CH)	5.91 (dt, 1H, J=10.2, 2.2 Hz)
12	35.2 (CH)	2.89 (mult, 1H)
13	40.7 (CH_2)	2.01 (dt, 1H, J=16.8, 6.6 Hz), 1.76 (br d, 1H, J=16.8 Hz)
14	66.7 (CH)	3.63 (mult, 1H)
15	45.6 (CH_2)	2.49 (dddd, 1H, J=15.4, 11.6, 4.7, 1.0 Hz), 1.80 (dt, 1H, J=15.4, 2.3 Hz)
16	72.8 (CH)	4.91 (mult, 1H)
17	41.2 (CH_2)	3.14 (ddd, 1H, J=18.8, 7.4, 1.0 Hz), 2.67 (dd, 1H, J=18.8, 0.8 Hz)
18	194.2 (Q)	

Structural Elucidation of the Macquarimicins

The molecular weight of 330 for macquarimicin A was determined by electron impact mass spectrometry (see Table 1). ^{13}C NMR and DEPT spectra[5] (see Table 2) of macquarimicin A indicated 19 carbon atoms with 21 attached protons and suggested the presence of functional groups including a ketone (δ 217.3 (Q)), an α,β-unsaturated ketone (δ 194.2 (Q), 136.4 (Q), 155.2 (CH)), an ester (δ 165.7 (Q)), an alcohol (δ 66.7 (CH)) and an olefin (δ 123.5 (CH), 127.7 (CH)). Thus the molecular weight could accommodate a molecular formula of $C_{19}H_{22}O_5$ containing four rings. 2-D Homonuclear and heteronuclear NMR experiments allowed connectivities for the assignment of a polyfunctional fused carbocycle (**1**) on which we have previously reported[2].

The molecular weight of 388 for macquarimicin B (**2**) was determined by electron impact mass spectrometry. ^{13}C NMR and DEPT spectra of macquarimicin B indicated 22 carbon atoms with 26 attached protons and suggested the presence of functional groups including two ketones (δ 220.6 (Q) and 214.5 (Q)), an ester (δ 167.3 (Q)), an alcohol (δ 67.9 (CH)), an olefin (δ 128.7 (CH), 123.0 (CH)) and an enol (δ 164.7 (Q) and 106.5 (Q)). A modified COSY, heteronuclear multiple-bond correlation HMBC[6] and heteronuclear multiple-quantum correlation HMQC[7] experiments indicated a structure for macquarimicin B clearly related to that of macquarimicin A. Macquarimicin B differed from A in containing an

enol functionality instead of a ketone. This could be accounted for by simple tautomerization. Additionally, macquarimicin B contained a methyl ketone, linked through a methylene at C-3 to the 10-membered carbocycle.

The structure of macquarimicin B was confirmed and the relative stereochemistry determined by a single crystal X-ray experiment. Macquarimicin B was crystalized from ethanol/water. The crystallographic data are summarized as follows: Monoclinic space group C2, a=22.402(5) Å, b=6.799(1) Å, c=15.619(3) Å, β=123.69(1)°, V=1979.3(7) Å^3, Z=4, Dcal=1.303 g/cm^3. The structure was solved with SHELX86[8]. The structure was refined by the full matrix least-squares method[9]. The final full-matrix least-squares with anisotropic temperature factors for all non-hydrogen atoms converged with an r factor of 0.048 (Rw=0.049, S=1.15). Fig. 1 is an ORTEP[10] drawing of macquarimicin B with thermal ellipsoids scaled at the 50% probability level for non-hydrogen atoms.

The molecular weight of 370 for macquarimicin C was determined by FAB positive ion mass spectrometry. ^{13}C NMR and DEPT spectra of macquarimicin indicated 22 carbon atoms with 26 attached protons and suggested the presence of functional groups including three ketones (δ 218.5 (Q), 206.7 (Q), 202.8 (Q)), an ester (δ 169.3 (Q)) and an olefin (δ 128.7 (CH), 126.0 (CH)). Thus the molecular weight could accommodate a molecular formula of $C_{22}H_{26}O_5$ containing five rings. 2-D Homonuclear and heteronuclear NMR experiments allowed connectivities for the assignment of a structure with portions similar to macquarimicin B, but containing an additional carbon-carbon bond linkage as designated in (**3**). This carbon-carbon linkage forming the additional carbocycle in macquarimicin C is defined predominantly by HMBC data in which long range coupling is observed

Fig. 1. ORTEP structure of macquarimicin B.

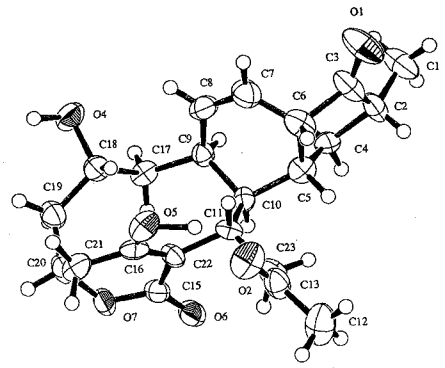


Table 3. ^{1}H NMR and ^{13}C NMR assignments for macquarimicin B (in $CDCl_3$).

Carbon No.	Carbon shift	Proton shift
1	167.3 (Q)	
2	106.5 (Q)	
3	38.3 (CH)	2.40 (mult, 1H)
3-CH_2	46.3 (CH_2)	3.42 (dd, 1H, J=19.9, 10.6 Hz), 2.95 (mult, 1H)
3-CO	214.5 (Q)	
3-CH_3	30.1 (CH_3)	2.14 (s, 3H)
4	38.0 (CH)	2.96 (mult, 1H)
5	36.5 (CH)	2.40 (mult, 1H)
6	34.5 (CH_2)	2.08 (mult, 1H), 1.42 (q, 1H, J=12.1 Hz)
7	43.5 (CH)	2.15 (mult, 1H)
7-CH_3	14.0 (CH_3)	1.10 (d, 3H, J=7.0 Hz)
8	220.6 (Q)	
9	46.4 (CH)	2.71 (mult, 1H)
10	123.0 (CH)	5.57 (dt, 1H, J=10.3, 3.2 Hz)
11	128.7 (CH)	5.94 (br d, 1H, J=10.3 Hz)
12	31.6 (CH)	2.83 (mult, 1H)
13	40.4 (CH_2)	1.90 (ddd, 1H, J=17.2, 9.5, 5.1 Hz), 1.86 (d, 1H, J=17.2 Hz)
14	67.9 (CH)	3.78 (mult, 1H)
15	45.3 (CH_2)	2.21 (mult, 1H), 1.67 (br d, 1H, J=13.2 Hz)
16	71.5 (CH)	4.54 (mult, 1H)
17	31.9 (CH_2)	2.93 (mult, 1H), 2.29 (d, 1H, J=18.3 Hz)
18	164.7 (Q)	

Table 4. ^{1}H NMR and ^{13}C NMR assignments for macquarimicin C (in $CDCl_3$).

Carbon No.	Carbon shift	Proton shift
1	169.3 (Q)	
2	65.9 (Q)	
3	28.4 (CH)	2.66 (ddd, 1H, J=11.1, 6.0, 2.0 Hz)
3-CH_2	48.4 (CH_2)	2.81 (dd, 1H, J=19.2, 6.0 Hz), 2.41 (dd, 1H, J=19.2, 2.0 Hz)
3-CO	206.7 (Q)	
3-CH_3	29.9 (CH_3)	2.13 (s, 3H)
4	37.0 (CH)	2.193 (mult, 1H)
5	34.6 (CH)	2.183 (mult, 1H)
6	34.1 (CH_2)	2.06 (mult, 1H), 1.38 (q, 1H, J=7.1 Hz)
7	43.6 (CH)	2.205 (mult, 1H)
7-CH_3	13.9 (CH_3)	1.06 (d, 3H, J=7.0 Hz)
8	218.5 (Q)	
9	46.3 (CH)	3.30 (mult, 1H)
10	126.0 (CH)	5.74 (dt, 1H, J=9.9, 3.3 Hz)
11	128.7 (CH)	5.34 (br d, 1H, J=9.9 Hz)
12	30.3 (CH)	2.50 (mult, 1H)
13	37.9 (CH_2)	1.90 (dt, 1H, J=13.7, 3.1 Hz), 1.62 (ddd, 1H, J=13.7, 5.9, 4.9 Hz)
14	31.9 (CH)	2.167 (mult, 1H)
15	33.4 (CH_2)	2.189 (mult, 1H), 1.85 (mult, 1H)
16	73.1 (CH)	4.98 (br s, 1H)
17	41.9 (CH_2)	2.70 (ddd, 1H, J=16.7, 3.3, 2.9 Hz), 2.45 (dd, 1H, J=16.7, 0.9 Hz)
18	202.8 (Q)	

between the quarternary carbon signal at δ 65.9 (Q) (C-2) and proton signals at δ 2.66 (C-3), 2.41 (C-3, CH_2), 2.167 (C-14) and 1.90 (C-13).

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