Chemistry of the Mycalamides, Antiviral and Antitumour Compounds from a Marine Sponge. Part 4.[1-3] Reactions of Mycalamide A and Alkyl Derivatives with Basic Nucleophiles

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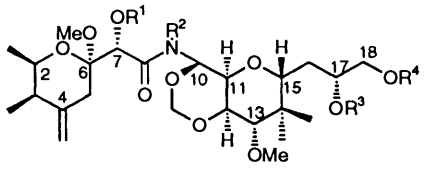
The chemistry of the potent antitumour and antiviral marine sponge metabolite, mycalamide A, and some of its alkyl derivatives, was examined by treatment with alkoxide, hydroxide and oxide bases in various solvents and with azide and hydride in dimethyl sulfoxide. The major rearrangement and cleavage products resulting from these reactions were characterised and tested for *in vitro* P388 anti-leukaemic activity.

Mycalamides A[1] **1** and B[2] **2** are potent antiviral and antitumour compounds isolated from a New Zealand marine sponge *Mycale* sp. We have recently described the preparation and biological properties of a series of acyl, alkyl and silyl derivatives as the first step in an extensive microscale structural modification study to determine structure–activity relationships.[3] That report established the importance of the central α-hydroxy amide portion for maintenance of biological potency. The initial experiments of this present study were conducted to determine the reactivity of the mycalamides towards basic reagents with the specific objective of hydrolysing the amide functionality and ascertaining the biological activities of the cleavage products.



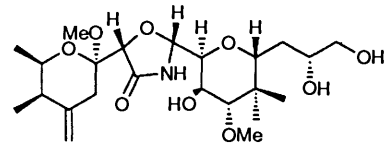
	R^1	R^2	R^3	R^4
1	H	H	H	H
2	H	H	Me	H
3	Me	Me	Me	Me
4	Me	H	H	H
5	CH_2Ph	H	H	H
6	CH_2Ph	H	H	CH_2Ph

Previous work had shown that mycalamide A was stable to amine bases and to aqueous potassium carbonate.[3] However treatment of mycalamide A with stronger bases and a variety of basic nucleophiles led to some unusual substitution, rearrangement, elimination and cleavage reactions, with no amide hydrolysis. These reagents included alkoxide, hydroxide and oxide bases in various solvents, together with azide and hydride ions in dimethyl sulfoxide (DMSO). We report here the characterisation of the new products derived from mycalamide A, and discuss their stereochemistries and possible mechanisms of formation.

Results and Discussion

Because of the limited supply of the mycalamides, all reactions were on a microscale, with typically only 0.5–2 mg of each product being obtained. Derivatives have been characterised by HRMS and NMR techniques. Wherever possible ^1H NMR spectral assignments have been verified by COSY and difference NOE methods, while in a few instances HMQC and HMBC[4] experiments have aided in the assignment of ^{13}C NMR data. Selected NMR spectroscopic data are presented in Tables 1 and 2.

Reaction of Mycalamide A with NaOMe–MeOH.—Mycalamide A **1** was treated with 1 mol dm^{-3} MeONa in MeOH at 50 °C to give equal amounts of two products. These products, only just resolved on silica gel TLC, were separated by analytical C_{18} reversed-phase HPLC, giving a 60% overall yield of purified products **7** and **8**. These products were isomers,



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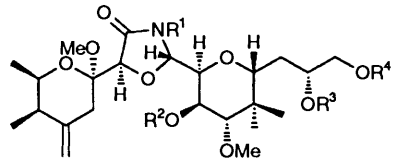


	R^1	R^2	R^3	R^4
8	H	H	H	H
9	COMe	COMe	COMe	COMe

having similar HRFABMS spectra, and molecular formulae $C_{23}H_{39}NO_9$. This represented a loss of CH_2O from mycalamide A **1**. The ^1H NMR spectra (Table 1) of each isomer were also similar, showing the loss of the two 10-OCH_2 doublets of mycalamide A, loss of the coupling between the 10-H and amide N-H protons, and the appearance of a new long-range coupling between the 7-H and 10-H protons. These data are consistent with the loss of formaldehyde from the C-10–C-12 methylene acetal of mycalamide A and with the presence of an oxazolidinone ring arising from the substitution of 7-O^- at C-10.[5] Further evidence for this oxazolidinone ring is given by the IR spectra of **7** and **8** in which the amide carbonyl stretch was about 30 cm^{-1} higher in frequency than for mycalamide A, consistent with the constrained *cis* geometry of a five-membered lactam compared with the normal open chain *trans*-amide.[6] Changes in the substitution of the C-7 and C-12 oxygens were confirmed by the acetylation of **8** (pyridine, acetic anhydride, 20 °C) to give the tetraacetate **9** (HRFABMS, ^1H NMR, ^{13}C NMR) [δ_H 2.0–2.5 (4 × 3 H); 12-H, 17-H, 18-H characteristic acetylation shift *ca.* +1 ppm].

The stereochemistries of the 2,5-substituents of the oxazo-

Table 1 ^{1}H NMR spectroscopic data for mycalamide A derivatives[a,b]

Proton	$\delta_H(J)$					
	7	**8**	**10**	**14**	**19**	**20**
2-H	3.95 (2.5, 6.6)	3.95 (2.6, 6.5)	3.79 (2.7, 6.6)	4.05 (2.7, 6.4)	3.96 (2.7, 6.6)	4.00 (2.7, 6.5)
2-Me	1.20 (6.6)	1.19 (6.6)	1.14 (6.6)	1.22 (6.5)	1.19 (6.6)	1.18 (6.6)
3-H	2.21 (2.2, 7.0)	2.22 (2.8, 7.0)	2.25 (2.2, 7.1)	2.23 (2.5, 7.0)	2.21 (2.5, 7.1)	2.23 (2.5, 7.0)
3-Me	1.05 (6.9)	1.04 (6.9)	0.98 (7.1)	1.06 (7.2)	0.95 (7.0)	0.98 (7.0)
4=CH(*Z*)	4.83 (1.9)	4.85 (2.0)	4.86 (2.1)	4.84 (1.8)	4.82 (1.8)	4.84 (1.9)
4=CH(*E*)	4.73 (1.9)	4.74 (1.9)	4.70 (2.1)	4.72 (1.9)	4.72 (1.8)	4.73 (2.0)
5a-H	2.40 (2.0, 13.8)	2.48 (1.9, 13.7)	2.44 (2.1, 14.4)	2.14 (2.0, 14.2)	2.31 (1.9, 14.4)	2.22 (2.0, 14.4)
5e-H	2.28 (13.9)	2.32 (13.7)	2.28 (14.2)	2.28 (14.2)	2.39 (14.3)	2.35 (14.2)
6-OMe	3.30	3.31	3.06	3.32	3.29	3.30
7-H	4.39 (1.5)	4.45 (2.4)		4.22 (2.7)	4.23	4.23
7-OH				3.86 (2.9)		
9-NH	6.58	7.87		8.30 (9.4)	8.56 (10.4)	7.13 (6.7)
10-H	5.59 (1.6, 8.9)	5.62 (2.3, 5.3)		5.41 (2.2, 9.3)	6.69 (2.0, 10.4)	3.75 (6.7)
10-OCH(*R*)				5.02 (6.7)		
10-OCH(*S*)				4.79 (6.6)		
11-H	3.88 (6.8, 8.9)	3.93 (5.4, 5.4)		3.78 (1.5, 2.0)		4.15 (6.7)
12-H	3.97 (6.7, 9.8)	4.00 (5.6, 7.4)		3.73 (1.5, 2.3)	4.20 (2.1, 9.0)	3.91 (6.7, 9.6)
13-H	2.96 (9.6)	2.98 (7.5)		2.92 (2.3)	2.76 (9.0)	2.91 (9.6)
13-OMe	3.61	3.56		3.38	3.62	3.58
14-Me(*R*)	0.98	1.03		1.21	0.96	0.97
14-Me(*S*)	0.89	0.88		0.94	0.93	0.87
15-H	3.60 (2.5, 9.8)	3.54 (2.2, 8.4)		3.66 (2.9, 11.7)	3.41 (2.8, 9.3)	3.63 (5.7, 7.3)
16-H	1.60 (2.5, 4.2, 14.6)	1.71 (1.2, 8.4, 15.2)		2.26 (7.8, 11.7, 15.6)	1.72 (m)	1.60 (m)
16-H	1.51 (6.9, 9.7, 14.6)	1.58 (2.3, 4.1, 15.2)		1.53 (2.9, 4.7, 15.4)	1.72 (m)	1.60 (m)
17-H	3.91 (m)	3.89 (m)		3.65 (m)	3.98 (m)	3.85 (m)
18-H	3.62 (6.5, 11.1)	3.59 (3.8, 11.2)		3.65 (3.2, 11.0)	3.70 (3.4, 11.0)	3.58 (3.8, 11.0)
18-H	3.56 (4.1, 11.2)	3.54 (6.7, 11.2)		3.48 (7.0, 11.0)	3.52 (7.3, 11.0)	3.47 (6.1, 11.2)

[a] Recorded in $CDCl_3$ with chemical shifts relative to $\delta_H(CHCl_3)$ 7.25 (coupling constants in Hz), except for **10** in D_2O, referenced to dioxane δ_H 3.70. [b] Full details of the spectroscopic results for compounds **5**, **6**, **9**, **11***E*, **11***Z*, **12**, **13**, **15**, **16**, **17** and **18** available.

Table 2 ^{13}C NMR spectroscopic data for mycalamide A derivatives[a]

Carbon	δ_C					
	7	**8**	**10**	**14**	**19**	**20**
C-2	69.31	69.29	70.69	69.70	69.81	69.45
2-Me	17.87	17.86	17.85	17.96	18.15	17.99
C-3	41.72	41.61	41.69	41.55	41.42	41.41
3-Me	11.70	11.80	12.20	11.84	11.82	12.08
C-4	146.07	146.37	148.03	145.80	145.90	145.64
4=CH_2	110.03	109.75	110.90	110.64	110.17	110.52
C-5	33.76	33.65	37.48	32.88	33.61	33.25
C-6	98.82	99.28	101.06	99.80	99.91	99.93
6-OMe	48.54	48.81	50.99	48.47	48.98	48.67
C-7	75.68	77.12	173.21	70.45	73.14	72.05
C-8	170.02	171.63		172.30	167.32	171.66
C-10	80.83	86.41		77.30	104.92	36.25
10-OCH_2				91.25		
C-11	77.58	74.12		61.05	141.65	74.51
C-12	68.89	68.89		72.35	69.60	69.45
C-13	88.02	86.88		83.51	90.72	86.78
13-OMe	63.09	61.73		59.31	62.59	62.67
C-14	41.50	39.81		36.62	41.46	41.15
14-Me(*R*)	23.42	24.43		27.08	22.89	23.70
14-Me(*S*)	13.78	16.28		22.89	13.74	14.42
C-15	78.58	79.56		81.54	85.01	77.75
C-16	31.44	31.01		30.00	31.79	31.41
C-17	72.04	72.48		70.45	71.63	71.86
C-18	66.74	66.76		66.24	66.61	66.75

[a] Recorded in $CDCl_3$ with chemical shifts relative to $\delta_C(CDCl_3)$ 77.01, except for **10** recorded in D_2O, referenced to dioxane δ_C 67.4

lidinone ring (C-7 and C-10) were determined as *cis* in **7** and *trans* in **8**, through the observation of NOE interactions between 7-H and 10-H for **7**, but not for isomer **8**. Furthermore, these *cis*- and *trans*-isomers both show long-range coupling between the 7-H and 10-H protons (1.5 Hz for **7** and 2.3 Hz for **8**), consistent with previous observations in which the larger coupling was associated with the *trans*-isomer.[7]

These oxazolidinone isomers must arise from an intramolecular substitution elimination reaction at C-10 in mycalamide A **1**. The stereochemistries of **7** and **8** therefore differ only at C-10, and this is supported by the ^{1}H and ^{13}C NMR chemical shift data (Tables 1 and 2). As the absolute stereochemistry of mycalamide A has been verified by synthesis,[8] isomer **8** has retained the C-10 configuration whereas isomer **7** is the product of inversion at C-10.

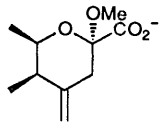
A number of other experiments were performed to investigate the mechanism responsible for formation of the observed products. There was no evidence for the inclusion of deuterium when the reaction was performed in CD_3OD so that there was no exchange of the 7-H or 10-H protons under these conditions. Treatment of each of the pure *cis*- and *trans*-isomers with MeONa in MeOH did not give interconversion of the two isomers, suggesting that the products were not equilibrating. However, reaction of each of the isomers with NaOH in aqueous MeOH gave isomers at C-7, consistent with previous results,[3] while the *trans*-isomer **8** could be converted into the *cis*-isomer **7** to the extent of about 60% as shown by ^{1}H NMR spectroscopy. The reaction of mycalamide A with BaO or KOH in DMSO also resulted in the formation of **7** and **8**, but in a ratio of about 5:2 favouring the *cis*-isomer **7**.

These results suggested the involvement of more than one reaction mechanism, depending on the nature of the solvent. The product due to inversion at C-10 (i.e. *cis*-**7**) should be formed exclusively under S_N2 conditions, and this is certainly the predominant product in DMSO, which is a less ionising solvent than MeOH. However some other mechanism is required to explain the presence of both isomers in the reaction mixture.

A common synthesis of 2,5-substituted-oxazolidin-4-ones involves the condensation of α-hydroxy amides or cyanohydrins with carbonyl compounds in the presence of an acid catalyst.[5,9]

Equilibrium processes have been proposed to account for yields favouring the thermodynamically more stable product.[7] An imine intermediate in the formation of these oxazolidinones has been disfavoured by Deavonport due to the low basicity of the amide nitrogen and an unfavourable geometry required for cyclisation.[7] However, such an intermediate could be formed from the amide anion of mycalamide A, which is known to exist under these conditions, by the cleavage of the C-10–O bond and donation of electron density from the amide nitrogen. Such intermediates have been proposed in reactions of related amide systems[10] and could account for the observed products, particularly under more ionising solvent conditions.

Reaction of Mycalamide A with BaO–Benzene.—The nucleophilic substitution reaction described above occurred readily in protic and dipolar aprotic solvents but would be disfavoured by the use of non-polar solvents such as benzene. Indeed, when mycalamide A was treated with barium oxide in benzene at 100 °C, one major water soluble product was isolated and identified as **10**. ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) showed only resonances from the intact O-1–C-6 tetrahydropyran ring portion, including the C-6 acetal, but the ^{13}C NMR chemical shifts [$\Delta\delta_C$ +3.3 (C-5), +1.2 (C-6)] and solubility properties were suggestive of a C-6 carboxylate salt. A negative ion FABMS spectrum showed an intense ion peak at *m/z* 199 (M^-) and a weaker peak at *m/z* 169 (M^- – MeOH). High resolution data were indicative of a molecular formula of $C_{10}H_{15}O_4$, consistent with the proposed structure.



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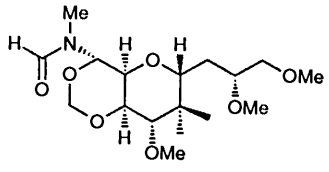

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Compound **10** evidently arises from a base-catalysed oxidative cleavage of mycalamide A between C-7 and C-8. However, no other fragments of the structure could be isolated. When the reaction was repeated at 80 °C, a ^{1}H NMR spectrum of the water-soluble fraction contained minor resonances, together with those of **10**, attributable to two components having the structure of mycalamide A from C-10 onwards. The exact structures of these minor products could not be determined, but they were evidently unstable towards base.

The formation of **7** and **8** above suggested that the C-10 position of mycalamide A could be a potential site for other substitution reactions under basic conditions. In order to investigate further this possibility it was necessary to consider the reactions of 7-*O*-protected mycalamide derivatives in base, or to consider reactions involving less basic nucleophiles, such as azide ion, on mycalamide A.

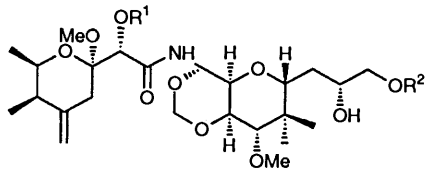
Reaction of 7,17,18-*Tri*-O-*methyl*-N-*methylmycalamide A with KOH–DMSO.*—The preparation of a fully methylated derivative, 7,17,18-tri-*O*-methyl-*N*-methylmycalamide A **3** following the procedure of Johnstone (KOH, MeI, DMSO, 20 °C) were described in a previous paper.[3] The treatment of **3** with powdered KOH in DMSO at 70 °C gave a single product, identified as **11**. The ^{1}H and ^{13}C NMR spectra contained resonances from only the right-hand portion of the starting structure **3**. Singlets at δ_H 8.25 and 8.16 in the ^{1}H NMR spectrum and signals at δ_C 164.1 and 163.6 in the ^{13}C NMR spectrum were attributed to a formyl group (C-8). The duplication of resonances in both spectra was characteristic of an *N*,*N*-disubstituted formamide structure undergoing classical slow conformational exchange [δ_H 3.00, 2.94 (N-Me), 6.09, 5.15 (10-H)]. NOE interactions between 8-H and N-Me and between 8′-H and 10′-H confirmed this behaviour.

An aqueous extract of the reaction product material contained no other fragments of the structure, as determined by ^{1}H NMR spectroscopy. It is therefore likely that the initially formed fragment derived from the left-hand portion of **3** underwent further rearrangement and decomposition reactions under the strongly basic conditions. Indeed, other reactions of **3** with MeONa in MeOH and NaOH in aqueous MeOH at elevated temperatures yielded no isolable products.

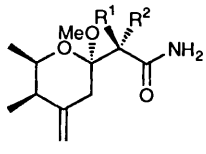
The observed cleavage of the C-6–C-8 masked β-dicarbonyl system may involve a base-catalysed 1,2-hydride shift from C-7 to C-8, with substitution of hydroxide ion at C-7. The resulting intermediate could then undergo normal reverse-aldol fragmentation to release the observed formamide.

It was noteworthy that the C-10 aza acetal system was stable towards base for *N*-methylated compounds. In order to examine further the reactivity of this system it was necessary to begin with a 7-O monoprotected mycalamide derivative. Since 7-*O*-methylmycalamide A[3] **4** was relatively difficult to prepare and purify, the synthesis of the less polar *O*-benzyl ether derivatives was examined.

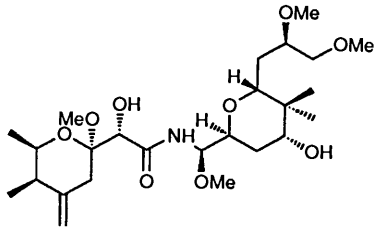
Reaction of 7-O-*Benzylmycalamide A with BaO–DMSO.*—When mycalamide A was treated with benzyl bromide and powdered KOH in DMSO at 20 °C (modified Johnstone methylation conditions[11]) mixtures of several mono-, di- and tri-benzylated derivatives were obtained readily. However, when BaO was substituted for KOH the above reaction was more selective, giving only mono- and di-*O*-benzyl derivatives (DCIMS). Separation by preparative TLC gave four compounds identified as 7-*O*-benzylmycalamide A **5** (HREIMS, ^{1}H



	R^1	R^2
12	CH_2Ph	H
13	CH_2Ph	CH_2Ph
14	H	H



	R^1	R^2
15	H	OH
16	H	OCH_2Ph
17	OCH_2Ph	H



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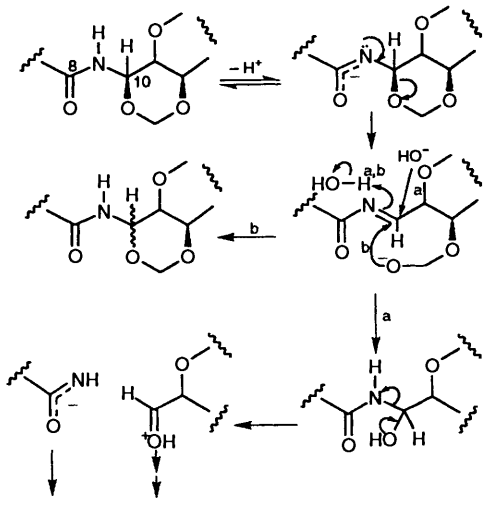
NMR, ^{13}C NMR) [δ_H 4.77, 4.68 (7-OCH$_2$Ph), $\Delta\delta_C$ +8.0 (C-7)], 7,18-di-*O*-benzylmycalamide A **6** (HRFABMS, ^{13}C NMR) [$\Delta\delta_C$ +7.3 (C-18)] and their C-10 epimers, (10*R*)-7-*O*-benzylmycalamide A **12** (HREIMS, NMR) and (10*R*)-7,18-di-*O*-benzylmycalamide A **13** (HRFABMS, NMR). The ^{1}H NMR spectrum of **12** showed large changes in the chemical shifts and coupling constants for substituents of the trioxadecalin ring system compared with those for **5** [$\Delta\delta_H$ +0.5 (N-H), −0.5 (10-H), −0.5 (12-H), −0.6 (13-H), −0.2 (13-OMe), +0.3 (14-Me*S*); $J_{H10-H11}$ (2.0), $J_{H11-H12}$ (1.8), $J_{H12-H13}$ (2.4) compared with **5** $J_{H10-H11}$ (10.0), $J_{H11-H12}$ (6.8) and $J_{H12-H13}$ (10.5)]. There were also (reciprocal) NOE interactions between the 10-H, 10-OCH and 12-H protons, between 13-H and both 14-Me groups and between 11-H and one 16-H which required that the trioxadecalin ring system of **12** adopt the opposite chair–chair conformation to that in mycalamide A (and **5**) and that C-10 be epimerised. Similarly, large shifts were observed in the ^{13}C NMR data for **5** and **12** [$\Delta\delta_C$ +3.0 (C-10), +4.8 (10-OCH$_2$), −9.9 (C-11), +4.5 (C-13), −5.2 (C-14), +4.4 (14-Me*R*), +9.2 (14-Me*S*)]. This conformational change for the C-10 epimers **12** and **13** allows the bulky amide sidechain to remain equatorial to the C-10 dioxane ring, thereby minimising unfavourable steric interactions.

Epimerisation at C-10 for certain 7-*O*-protected derivatives under basic conditions was also observed during the total synthesis of mycalamides A and B.[8] Further experiments were therefore performed to investigate this epimerisation. For example, when 7-*O*-benzylmycalamide A **5** was treated with BaO in DMSO at 70 °C, a 1:1 mixture of **5** and **12** was obtained. The reaction of (10*R*)-7-*O*-benzylmycalamide A **12** with MeONa in MeOD gave products which were epimeric at C-7 and/or C-10, with incorporation of deuterium only at C-7, not C-10. Furthermore, no epimerisation at C-10 occurred for *N*-alkylated mycalamide derivatives under basic conditions. These results seem to exclude mechanisms involving a direct exchange of 10-H, and suggest the involvement of an imine intermediate by cleavage of the C-10–O bond (Scheme 1). Support for this is seen in the racemisation of α-acetamide-α-vanillylpropiononitrile by NaCN in DMSO which was shown to proceed *via* an analogous imine intermediate generated by the expulsion of CN$^-$ from the amide anion, and occurred only for 2° amides.[12]

Further treatment of the original benzylation product mixture (containing compounds **5**, **6**, **12** and **13**) with BaO in DMSO at 80 °C gave complete conversion into two new products in almost equal proportions. These were separated by reversed-phase HPLC phase and identified as 7-*O*-benzylpederamide **16** and its epimer (7*R*)-7-*O*-benzylpederamide **17**. The isomeric nature of these compounds followed from their identical molecular formula of $C_{18}H_{25}NO_4$ (HREIMS). The 7-OH primary amide, pederamide **15**, had previously been obtained by acid-catalysed cleavage of pederin **18**,[13,14] and was the target of several syntheses.[15,16] A comparison of the ^{1}H NMR data for **16** with data reported for **15** revealed a good agreement, allowing for the presence of a benzyl ether group at C-7 in **16**. Data for **17** were similar to those for **16**, but differences in chemical shifts for resonances relating to the C-5–C-7 portion of the structure were characteristic of an epimerisation at C-7 [$\Delta\delta_H$ +0.3 (5a-H), −0.3 (5e-H), −0.3 (6-OMe), +0.1 (7-H); $\Delta\delta_C$ +1.9 (C-7), +1.3 (7-OCH$_2$)] as observed for methylated mycalamides under basic conditions.[3] No comparable cleavage reactions were found for any mycalamide derivatives containing an *N*-alkyl group.

This base-catalysed cleavage of the C-10–N bond of 7-*O*-benzylmycalamide A derivatives (**5**, **6**, **12**, **13**), but not of any *N*-alkylated derivatives, is consistent with a hydrolysis of the proposed imine intermediate (Scheme 1). A substitution of hydroxide ion at C-10 gives an unstable hemiaminal, which can



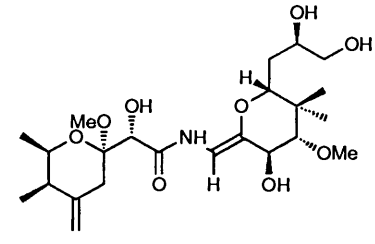
Scheme 1 Proposed mechanism involving an imine intermediate in the N-9–C-10 cleavage (pathway a) and C-10 epimerisation (pathway b) reactions of 7-*O*-alkylated mycalamide derivatives

cleave to form the observed primary amide and an aldehyde. The fact that no stable fragments of the right hand portions of compounds **5**, **6**, **12** and **13** were isolated is consistent with the reactivity of such compounds (aldehydes) towards base.

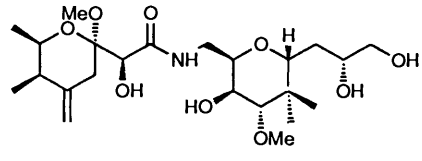
The presence of an ether group at C-7 instead of a free hydroxy was not favourable for the determination of structure–activity correlations for the above derivatives, since the deactivating effect of any 7-*O* substituent had already been noted.[3] Therefore the reactions of mycalamide A with other nucleophiles such as azide and hydride ions were considered.

Reaction of Mycalamide A with Azide–DMSO.—The reaction of mycalamide A with sodium azide in DMSO at 140 °C gave a mixture of products, which were separated by preparative silica gel TLC, followed by reversed-phase HPLC.

The major product, **19**, was isolated in 48% purified yield.



19



20

HRFABMS indicated a molecular formula of $C_{23}H_{39}NO_9$, corresponding to a loss of CH_2O from mycalamide A. The ^{1}H NMR spectrum (data in Table 1) showed an absence of the 10-OCH$_2$ and 11-H resonances and the appearance of a long-range coupling between 10-H and 12-H of 2.0 Hz. These results, together with changes in the ^{1}H chemical shifts [$\Delta\delta_H$ +1.1 (N-H), +0.8 (10-H), −0.7 (13-H), −0.2 (15-H)] and the observed ^{13}C NMR data (Table 2) [δ_C 104.9 (C-10), 141.7 (C-11); $\Delta\delta_C$ −4.5 (C-8), −4.8 (C-12), +11.6 (C-13), +6.0 (C-15)] were consistent with the structure **19** having a double

bond between C-10 and C-11. The observation of NOE interactions between the 10-H and 12-H protons, and between the N-H, 15-H and 17-H protons, established the *Z*-stereochemistry for the double bond in **19**.

Of the remaining products isolated from the reaction the most dominant (12% yield) was identified as (10*R*)-mycalamide A **14** (HRFABMS, ^{1}H NMR). The ^{1}H NMR data obtained for **14** (Table 1) were similar to those for (10*R*)-7-*O*-benzylmycalamide A **12**, except in the vicinity of C-7 [$\Delta\delta_H$ −0.3 (5a-H), +0.2 (7-H), +0.5 (N-H)]. NOE experiments also gave results similar to those for **12**, confirming the inversion of configuration at C-10 and the related conformational change for the trioxadecalin ring system. (10*R*)-Mycalamide A **14** was less polar than mycalamide A by reversed-phase HPLC but the two were coincident on silica gel TLC.

Mycalamide A oxazolidinones **7** and **8** were also identified as minor products from this reaction (3 and 10% yields respectively). Another minor product (8% yield) was pederamide **15** (HREIMS, ^{1}H NMR). Pederamide was the major product (83% yield) when the elimination product **19** was treated with sodium azide in DMSO as above.

In these reactions in DMSO, the azide ion is behaving as a significantly strong base, enabling the formation of the amide and C-7 hydroxy anions and assisting in the removal of 11-H. This basicity is entirely consistent with the reported properties of azide ion in dipolar aprotic solvents.[17] The fact that no substitution products were observed may be explained by several factors, including the hindered reaction site, the high reaction temperature which favours elimination reactions and the relatively low concentration of free nucleophilic azide ion in solution. The presence of trace amounts of water in the solvent may also account for the formation of the hydrolysis product pederamide **15** in this reaction.

Reaction of Mycalamide A with $NaBH_4$–DMSO.—It was proposed that the observed reactivity of the C-10 aza-acetal under basic conditions could permit a reductive displacement at C-10 with hydride ion. Sodium borohydride in dipolar aprotic solvents is an excellent source of nucleophilic hydride ion, and has been successfully employed on various halide and sulfonate ester substrates.[18]

The reaction of mycalamide A with an excess of $NaBH_4$ in DMSO at 130 °C gave a mixture of products which were separated by silica gel TLC. The major product was the *trans*-oxazolidinone **8** (33% yield), with the *cis*-isomer **7** being a minor product (13% yield). The other major product, isolated in about 20% yield, was identified as the C-10 reduction product **20**.

An HRFABMS spectrum of **20** indicated a molecular formula of $C_{23}H_{41}NO_9$, corresponding to a loss of CO from mycalamide A. An FT-IR spectrum showed that the amide carbonyl stretch was 20 cm^{-1} lower in frequency than for mycalamide A, consistent with the removal of the electron-withdrawing C-10 oxygen substituent. The ^{1}H NMR spectrum (data in Table 1) showed the loss of the 10-OCH_2 acetal, changes in the multiplicities of the N-H (d to t) and 11-H (dd to q) resonances and the appearance of a two proton triplet (δ 3.75) for the two 10-H protons instead of the usual one proton triplet. Changes in the proton chemical shifts [$\Delta\delta_H$ −0.4 (N-H), +0.3 (11-H), −0.3 (12-H), −0.6 (13-H)] and the carbon chemical shifts (Table 2) [$\Delta\delta_C$ −37.4 (C-10), +3.3 (C-11), −5.0 (C-12), +7.6 (C-13)] were also consistent with the structure **20**.

Biological Activity.—The biological activities of all new derivatives are recorded as IC_{50} values (ng cm^{-3}) against the murine leukaemia P388 cell line in Table 3. Earlier studies had shown that methylation of the 7-OH group of mycalamide A caused an approximately 10^2-fold reduction in activity.[3] Thus it was not surprising that the oxazolidinones **7**–**9** and the 7-*O*-benzyl derivatives **5**, **6**, **12** and **13** were found to have poor activities. The relative inactivity of the cleavage fragments **10**, **11** and **15** demonstrates further that both segments of the mycalamide structure are essential to the biological activity. Of the remaining products **14**, **19** and **20**, only the reduction product **20** (40-fold deactivation) had significant biological activity, showing that the C-10 acetal was important and that the C-10 configuration was crucial for activity to be displayed. These observations reinforce the conclusions of an earlier study[3] that the centrally located α-hydroxyamidoacetal functionality is vitally important for the biological activity of the mycalamides.

Table 3 IC_{50} Values of mycalamide derivatives against P388 cells

Compound	IC_{50}/ng cm^{-3}
1	0.5
2	0.1
3	2000
4	80
5	90
6	170
7	300
8	400
9	5000
10	110
11	800
12	600
13	1300
14	300
15	7500
16	800
17	800
19	1500
20	20

Experimental

General Procedure.—The same general procedure was used as described previously.[3] In addition, FT-IR spectra were recorded from sample films on KBr plates using a Perkin-Elmer 1600 series spectrometer or a Bio-Rad Digilab Division FTS-40 spectrometer. NMR spectra were recorded on a Varian XL300 spectrometer, operating at 300 MHz for ^{1}H and at 75 MHz for ^{13}C nuclei, except for some HMQC and HMBC data, which were recorded on a VXR500S spectrometer operating at 500 MHz for ^{1}H. Mycalamide A **1** and B **2** were obtained from a *Mycale* sp. sponge as described previously.[1,2]

WARNING: The mycalamides and several of their derivatives cause severe dermatitic reactions if allowed to come into contact with the skin.

Preparation of Mycalamide A Oxazolidinones.—A solution of mycalamide A **1** (3.8 mg) in sodium methoxide–methanol (1 mol dm^{-3}; 0.3 cm^3) was stirred at 50 °C for 8 h. The solvent was removed and the residue extracted with $CHCl_3$ (3 × 1 cm^3). Filtration and removal of the solvent gave a crude product (3.3 mg) which was a mixture of two components (TLC, HPLC). Preparative reversed-phase HPLC (45% water in methanol) gave two fractions. (i) *Mycalamide A* cis-*oxazolidinone* **7** as an oil (1.0 mg); ν_{max}(film)/cm^{-1} 3700–3100, 3073, 2964, 2926, 2855, 1718, 1430, 1380, 1260, 1102, 1071, 1045, 1018 and 800; ^{1}H and ^{13}C NMR data in Tables 1 and 2 (HRFABMS. MNa^+, 496.2527. *M*Na, 496.2523); DCIMS (NH_3) *m*/*z* 491 (MNH_4^+, 5%), 461 (6), 460 (30), 459 (MNH_4^+ − MeOH, 100), 443 (17) and 442 (MH^+ − MeOH, 63). (ii) *Mycalamide A* trans-

oxazolidinone **8** as an oil (1.3 mg); $\nu_{max}(CHCl_3)/cm^{-1}$ 3600–3200, 2920, 1715, 1600, 1400 and 1100–1020; ^1H and ^{13}C NMR data in Tables 1 and 2 (HRFABMS. MNa^+, 496.2539. *M*Na, 496.2523); DCIMS (NH_3) *m/z* 461 (6%), 460 (29), 459 (MNH_4^+ − MeOH, 100), 443 (7) and 442 (MH^+ − MeOH, 41).

Preparation of Acetate Ester **9**.—A solution of mycalamide A *trans*-oxazolidinone **8** (1.7 mg) in pyridine (0.15 cm^3) and acetic anhydride (0.15 cm^3) was stirred at room temp. for 2 d. The solution was concentrated under nitrogen then water (2 cm^3) was added and the mixture extracted with CH_2Cl_2 (3 × 2 cm^3). The solvent was removed to give N-*acetylmycalamide A* trans-*oxazolidinone triacetate* **9** as an oil (1.8 mg); ν_{max}(film)/cm^{-1} 3075, 2968, 2925, 2850, 1745, 1715, 1657, 1438, 1373, 1295, 1264, 1228, 1144, 1094 and 1047; ^1H and ^{13}C NMR data in Supplementary Tables 1 and 3* (HRFABMS. MNa^+, 664.2982. *M*Na, 664.2945).

Reaction of Mycalamide A with BaO–Benzene.—A solution of mycalamide A **1** (3 mg) and BaO (20 mg) in benzene (0.4 cm^3) was stirred in a sealed vial at 100 °C for 20 h. The solvent was removed and the residue partitioned in water–chloroform (1 : 1) (5 cm^3). The aqueous portion was washed with $CHCl_3$, then the solvent removed to give a mixture of inorganic salts and the *carboxylate salt* **10** as a solid (14 mg); ^1H and ^{13}C NMR data in Tables 1 and 2 (HRFABMS. M^-, 199.0965. *M*, 199.0970). The chloroform-soluble material (1 mg) was separated by preparative reversed-phase HPLC (32% water in methanol) into two fractions. (i) Mixture (0.5 mg) which contained unchanged mycalamide A **1**. (ii) (10R)-*Mycalamide A* **14** (0.3 mg) which was subsequently characterised after its alternative preparation below.

Reaction of 7,17,18-*Tri*-O-*methyl*-N-*methylmycalamide A with KOH–DMSO*.—A solution of 7,17,18-tri-*O*-methyl-*N*-methylmycalamide A **3** (3.9 mg) in DMSO (0.2 cm^3) was prepared from mycalamide B **2** (3.6 mg), powdered KOH (6 mg) and MeI (4 mm^3) in DMSO.[3] Powdered KOH (2 mg) was added, then the solution was stirred at 70 °C for 5 h. Water (0.5 cm^3) was added and the mixture extracted with chloroform (3 × 0.3 cm^3). The solvent was removed to give the N-*formyl fragment* **11** as an oil (1.8 mg); $\nu_{max}(CHCl_3)/cm^{-1}$ 2900, 2860, 1680, 1600, 1380 and 1080–1020; ^1H and ^{13}C NMR data in Supplementary Tables 1 and 3* [HRCIMS (C_4H_{10}). MH^+, 362.2179. *M*H, 362.2179].

Preparation of O-*Benzyl Derivatives*.—A solution of mycalamide A **1** (6 mg), BaO (26 mg) and benzyl bromide (10 mm^3) in DMSO (0.3 cm^3) was stirred at 60 °C for 2 h. Water (0.5 cm^3) was added and the mixture transferred onto a reversed-phase column (200 mg C_{18}, equilibrated to water), which was then flushed with water (8 cm^3) and eluted with methanol (6 cm^3). The methanol fraction was evaporated to dryness and subjected to preparative silica gel TLC [light petroleum–ethyl acetate (1 : 2)]. Four bands of silica were recovered and eluted with ethyl acetate to give four fractions. (i) 7-O-*benzylmycalamide A* **5** as an oil (1.0 mg); ^1H and ^{13}C NMR data in Supplementary Tables 1 and 3* (HREIMS. M^+ − MeOH, 561.2919. *M* − MeOH, 561.2938); DCIMS (NH_3) *m/z* 611 (MNH_4^+, 7%), 582 (8), 581 (26), 580 (34), 579 (MNH_4^+ − MeOH, 100), 564 (5), 563 (6) and 562 (MH^+ − MeOH, 17). (ii) (10R)-7-O-*Benzylmycalamide A* **12** as an oil (1.0 mg); ^1H and ^{13}C NMR data in Supplementary Tables 1 and 3; DEIMS: *m/z* 593 (M^+, 1%) and 562 (M^+ −OCH_3, 4%).

Structural proof for compound **12** relies on NMR and LR mass spectrometric data since satisfactory HR data could not be obtained. DCIMS (NH_3) *m/z* 611 (MNH_4^+, 2%), 594 (MH^+, 3), 582 (9), 581 (26), 580 (28), 579 (MNH_4^+ − MeOH, 80), 565 (12), 564 (37), 563 (33) and 562 (MH^+ − MeOH, 100). (iii) 7,18-*Di*-O-*benzylmycalamide A* **6** as an oil (1.5 mg); ^1H and ^{13}C NMR data in Supplementary Tables 1 and 3* (HRFABMS. MH^+ − MeOH, 652.3510. *M*H − MeOH, 652.3486); DCIMS (NH_3) *m/z* 703 (7%), 702 (12), 701 (MNH_4^+, 28), 672 (9), 671 (27), 670 (41), 669 (MNH_4^+ − MeOH, 100), 654 (5), 653 (8) and 652 (MH^+ − MeOH, 19). (iv) (10R)-7,18-*Di*-O-*benzylmycalamide A* **13** as an oil (1.5 mg); ^1H and ^{13}C NMR data in Supplementary Tables 1 and 3* (HRFABMS. MH^+ − MeOH, 652.3495. *M*H −MeOH, 652.3486); DCIMS (NH_3) *m/z* 701 (MNH_4^+, 4%), 672 (5), 671 (18), 670 (42), 669 (MNH_4^+ − MeOH, 100), 654 (11), 653 (21) and 652 (MH^+ − MeOH, 53).

Reaction of 7-O-*Benzyl Derivatives with BaO–DMSO*.—A solution of mycalamide A **1** (4 mg), BaO (20 mg) and benzyl bromide (9 mm^3) in DMSO (0.3 cm^3) was stirred at 65 °C overnight. BaO (10 mg) was added and the mixture stirred at 80 °C for 1 d. Work-up as above gave a mixture of two compounds (HPLC). Preparative reversed-phase HPLC (25% water in methanol) gave two fractions. (i) 7-O-*Benzylpederamide* **16** as an oil (1.0 mg); ν_{max}(film)/cm^{-1} 3464, 3338, 3203, 3070, 3031, 2972, 2927, 2855, 1685, 1607, 1490, 1455, 1379, 1325, 1228, 1146, 1102, 1075, 1048 and 1015; ^1H and ^{13}C NMR data in Supplementary Tables 2 and 4* (HREIMS. M^+, 319.1788. *M*, 319.1784). (ii) (7R)-7-O-*Benzylpederamide* **17** as an oil (1.0 mg); ν_{max}(film)/cm^{-1} 3474, 3360, 3069, 3031, 2972, 2922, 2851, 1686, 1587, 1456, 1378, 1325, 1231, 1144, 1077, 1041 and 1028; ^1H and ^{13}C NMR data in Supplementary Tables 2 and 4* (HREIMS. M^+, 319.1797. *M*, 319.1784).

Reaction of Mycalamide A with Azide–DMSO.—A solution of mycalamide A **1** (6 mg) and sodium azide (26 mg) in DMSO (0.3 cm^3) was stirred at 135 °C for 4 d. Water (0.6 cm^3) was added and the mixture transferred onto a reversed-phase column and eluted with water and methanol as above. The methanol fraction was separated by preparative silica gel TLC (2 × ethyl acetate) into five fractions. (i) (11R)-*Mycalamide A* trans-*oxazolidinone* (incompletely characterised) as an oil (0.4 mg). (ii) Z-Δ^{10}-10-*Deformylmycalamide A* **19** as an oil (2.8 mg); ν_{max}(film)/cm^{-1} 3400, 3073, 2971, 2929, 2855, 1670, 1512, 1380, 1295, 1228, 1092, 1074, 1043 and 1015; ^1H and ^{13}C NMR data in Tables 1 and 2 (HRFABMS. MH^+ − MeOH, 442.2444. *M*H − MeOH, 442.2441). (iii) Mixture (0.7 mg) of mycalamide A **1**, mycalamide A *cis*-oxazolidinone **7** and mycalamide A *trans*-oxazolidinone **8**. (iv) Mixture (1.7 mg) which was further purified by preparative reversed-phase HPLC (45% water in methanol) to give (E)-Δ^{10}-10-*deformylmycalamide A* (incompletely characterised) as an oil (0.3 mg) and (10*R*)-*mycalamide A* **14** as an oil (0.7 mg); ν_{max}(film)/cm^{-1} 3357, 2923, 2853, 1688, 1531, 1460, 1380, 1197, 1140, 1099, 1075, 1035 and 1018; ^1H and ^{13}C NMR data in Tables 1 and 2 (HRFABMS. MK^+, 542.2339. *M*K, 542.2368). (v) Pederamide **15** (0.2 mg), subsequently characterised after its alternative preparation below.

Reaction of Z-Δ^{10}-10-*Deformylmycalamide A with Azide–DMSO*.—A solution of Z-Δ^{10}10-deformylmycalamide A **19** (2.0 mg) and sodium azide (23 mg) in DMSO (0.3 cm^3) was stirred at 90 °C for 6 d. Work-up as above yielded a mixture which was separated by preparative silica gel TLC (2 × ethyl acetate) into two fractions. (i) Z-Δ^{10}-10-deformylmycalamide A **19** (0.5 mg). (ii) Pederamide **15** as an oil (0.6 mg); ν_{max}(film)/cm^{-1} 3484, 3358, 3068, 2979, 2914, 2851, 1683, 1573, 1417, 1375, 1323, 1230, 1139, 1124, 1103, 1071, 1039 and 1015;

* Supplementary publication no. 56992 (4 pages). For details of the supplementary publications scheme, see Instructions for Authors (1994), *J. Chem. Soc., Perkin Trans. 1*, Issue 1.

^1H and ^{13}C NMR data in Supplementary Tables 2 and 4* (HREIMS. M^+ – MeOH, 197.1049. *M* – MeOH, 197.1052).

Reaction of Mycalamide A with $NaBH_4$–DMSO.—A solution of mycalamide A **1** (6 mg) and $NaBH_4$ (24 mg) in DMSO (0.3 cm^3) was stirred at 130 °C for 2 d. Water (0.6 cm^3) was added and the mixture transferred onto a reversed-phase column and eluted with water and methanol as above. The methanol fraction was separated by preparative silica gel TLC [2 × ethanol–ethyl acetate (1:25)] into five fractions. (i) 10,12-O-*Dihydromycalamide A* **20** as an oil (0.9 mg); ν_{max}(film)/cm^{-1} 3700–3100, 2961, 2925, 2874, 1660, 1548, 1458, 1382, 1262, 1101, 1073, 1044 and 1014; ^1H and ^{13}C NMR data in Tables 1 and 2 (HRFABMS. MH^+ – MeOH, 444.2614. *M*H – MeOH, 444.2597). (ii) Δ^4-*Mycalamide A* trans-*oxazolidinone* (incompletely characterised) as an oil (0.3 mg). (iii) Mixture (1.2 mg) of mycalamide A **1** and mycalamide A *cis*-oxazolidinone **7**. (iv) Mixture (1.2 mg) of mycalamide A **1** and mycalamide A *trans*-oxazolidinone **8**. (v) Mycalamide A *trans*-oxazolidinone **8** (1.0 mg).

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