PRODUCTION BY CONTROLLED BIOSYNTHESIS OF A NOVEL IONOPHORE ANTIBIOTIC, CEZOMYCIN (DEMETHYLAMINO A23187)

Sir:

The present note reports the preparation by a method of controlled biosynthesis of a novel A23187 analog, demethylamino A23187, which we have named cezomycin.

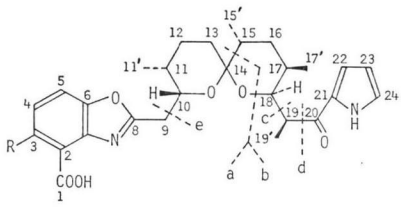
A23187[1] (**1a**, Fig. 1) is one of a very small group of ionophore antibiotics able to transport alkaline-earth cations across biological membranes[2]. By its chemical structure it belongs, with X-14547[3], to the naturally-occurring carboxylic pyrrole polyether series of antibiotics[4]. Preparation of analogs of A23187 is likely to help elucidate its mode of action by revealing which structural factors are implicated in its ionophore properties and biological activity (G. JEMINET, personal communication). ABBOTT *et al.*[5] had prepared *N*-demethylated A23187, 16-hydroxy-*N*-demethyl A23187 and 16-hydroxy A23187 by microbiological transformation of A23187 methyl ester using a strain of *Streptomyces chartreusis* NRRL 11407 found among 200 randomly selected soil organisms.

A23187 was obtained from *Streptomyces chartreusis* NRRL 3882. The culture medium consisted of glucose 10 g, Casamino acids 4 g, yeast extract 1 g and meat extract 1 g per liter of medium. The pH was adjusted to 7.2 before sterilizing. It was found that addition of L-tryptophan (1 g/liter at 24 hours) to this culture medium inhibited production of A23187 (40 mg/liter) and led to the formation of cezomycin (**1b** Fig. 1) (100~150 mg/liter). Spectroscopic data showed this product to be different from A 23187 in lacking the 3-methylamino group.

a) The mass spectrum determined on a V. G. Micromass 70.70 F instrument (electron impact 70 eV) showed major peaks indicated in Fig. 1 including one at 494 (62%) which corresponds to the molecular mass of A23187 (523 less $NHCH_3$) and one at 177 corresponding to $C_9H_6NO_3^+$ (3-unsubstituted benzoxazole).

b) The ^1H NMR spectrum (CAMECA 350 in $CDCl_3$) of cezomycin did not show either a methyl signal at δ 2.95 ppm or that expected of the nitrogen-bound hydrogen (δ 8.1 ppm) of the methylamine group of A23187.

Fig. 1. Main fragmentations cezomycin mass spectrum.



1a R = $NHCH_3$ A23187
1b R = H Cezomycin

m/z 494 $C_{28}H_{34}N_2O_6$
a → *m/z* 290 $\xrightarrow{e}$ *m/z* 177
b → *m/z* 205 $\xrightarrow{c}$ *m/z* 123 $\xrightarrow{d}$ *m/z* 94

c) The ^{13}C NMR spectrum (Brucker CPX 200) of cezomycin (Table 1) is compared with that of A23187 whose signals have been assigned elsewhere[6]. The signal at 30 ppm due to the nitrogen-bound methyl of A23187 is absent. Peak assignments for cezomycin are proposed in Table 1. The replacement of the 3-CH_3NH group by H has little influence on the *meta*-position carbons 5 and 7, but markedly shifts the peaks due to the other aromatic carbons.

Inhibition of A23187 biosynthesis and favored formation of cezomycin may be explained thus: The benzoxazole moiety of A23187 is formed from 3-hydroxyanthranilic acid (HAA) itself formed from tryptophan *via* a classical metabolic pathway involving 3-hydroxy kynurenin. HAA is converted into 6-methylamino 3-hydroxy anthranilic acid (MAHAA) by hydroxylation, transamination, and then methylation of the 6-amine. A23187 would then be formed from MAHAA and a "carboxylic acid synthon" bearing the spiroketal and pyrrole functions.

Supplementary tryptophan in the medium (1 g/liter) is metabolized not only to give HAA but also large amounts of anthranilic acid. Under these conditions, the latter could hinder hydroxylation of HAA by competitive inhibition. The carboxylic acid synthon would then couple with HAA itself.

Addition of anthranilic acid (0.5 g/liter) was indeed found to inhibit A23187 biosynthesis and favor production of cezomycin. Addition of anthranilic acid (0.5 g/liter) and HAA (0.5 g/liter) resulted in normal production of A23187.

The formation of complex cezomycin-Ca in methanol were pointed out using the UV spectro-

Table 1. ^{13}C NMR data[a].

A23187		Cezomycin	
Assignment	Chemical shift (ppm)/TMS	Assignment	
C-20	193.7	194.4 (s)	C-20
C-1	168.1	167.8 (s)	C-1
C-8	166.1	165.4 (s)	C-8
C-3	150.8		
C-6	141.7	150.6 (s)	C-6 C *para* to C-3
C-7	140.8	140.8 (s)	C-7 C *meta* to C-3
C-21	133.0	133.3 (s)	C-21
		126.8 (d)	C-3 without $N-CH_3$
		125.0 (d)	C-4 C *ortho* to C-3
C-24	124.3	124.8 (d)	C-24
		120.4 (s)	C-2 C *ortho* to C-3
C-5	116.7	117.1 (d)	C-5 C *meta* to C-3
C-22	116.3	115.2 (d)	C-22
C-23	110.1	110.3 (d)	C-23
C-4	108.4		
C-14	98.5	98.4 (s)	C-14
C-2	98.2		
C-18	72.9	73.1 (d)	C-18
C-10	68.4	68.8 (d)	C-10
C-19	42.5	42.6 (d)	C-19
C-16	35.2	35.3 (t)	C-16
C-9	32.4	32.7 (t)	C-9
C-15	32.3	32.4 (d)	C-15
$N-CH_3$	30.0		
C-11	28.8	29.3 (d)	C-11
C-17	28.3	28.4 (d)	C-17
C-12	25.7	25.8 (t)	C-12
C-13	25.4	25.5 (t)	C-13
C-15′	16.1	16.2 (q)	C-15′
C-19′	13.0	12.9 (q)	C-19′
C-11′	11.3	11.4 (q)	C-11′
C-17′	10.7	10.9 (q)	C-17′

[a] SFOR multiplicities are indicated in parenthesis.

scopic method. The determination of equilibria constants are in progress. Calcium transport by A23187 across a chloroformic membrane has been studied[7]. A preliminary study on cezomycin indicates a similar activity.

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