Biosynthesis of Asukamycin. Formation of the 2-Amino-3-hydroxycyclopent-2-enone Moiety

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Feeding experiments with labelled precursors suggest that the 2-amino-3-hydroxycyclopent-2-enone moiety of the anticoccidial antibiotic, asukamycin, is formed by a novel intramolecular cyclization of 5-aminolevulinic acid.

A number of antibiotics have been discovered in recent years which contain as a unique structural feature a 2-amino-3-hydroxycyclopent-2-enone (**1**) moiety. These include in addition to asukamycin (**2**),[1,2] the subject of this report, the related compounds manumycin[3] and U-56,407,[4] as well as reductiomycin,[5] moenomycin A,[6] senacarcin A,[7] virustomycin A,[8] and bafilomycin.[9] We report results which suggest a mode of biosynthesis of this novel structural unit.

Experiments were carried out using cultures of *Streptomyces nodosus* subsp. *asukaensis* grown in 100 ml of medium (2% glucose, 2% soybean meal, 0.3% NaCl, pH adjusted to 7.0) in 500 ml indented Erlenmeyer flasks at 28 °C on a rotary shaker (300 r.p.m.). Precursors were fed 24 h after inoculation and 48 h later asukamycin (**2**) was extracted with ethyl acetate and purified by preparative layer chromatography (silica gel; benzene–acetone, 3 : 1). The purified (**2**) was quantitated by h.p.l.c. (μ-Bondapak C_{18}; MeOH–H_2O, 3 : 1) and subjected to liquid scintillation counting (Beckman LS7500) and/or ^{13}C n.m.r. spectroscopy (Bruker WM-300).

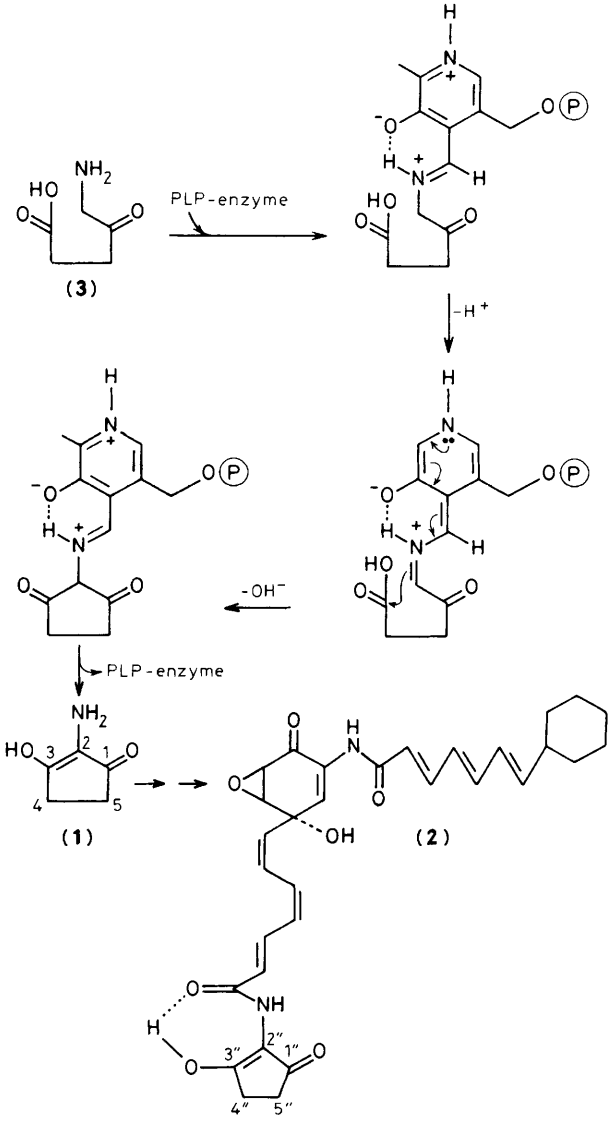
Inspection of the structure of (**1**) suggests that C-1 and the attached nitrogen may be derived from glycine. In agreement with this notion, [2-^{14}C]glycine gave specific incorporations into (**2**) ranging from 2 to 21%, depending on the amount of material fed (Table 1, expts. 1—4). Incorporation of glycine specifically into the (**1**) moiety was demonstrated by feeding [2-^{13}C,^{15}N]glycine (500 mg; 90 atom % ^{13}C, 99 atom % ^{15}N; 10 flasks). The signal for C-2″ in the ^{13}C n.m.r. spectrum of the resulting (**2**) (δ 115.39) appears as a 3-line pattern (J_{C-N} 17.6 ± 0.6 Hz) (Figure 1), indicating predominantly intact incorporation of the C-2–N assembly of glycine into (**1**).

It occurred to us that (**1**) contains the entire carbon–nitrogen skeleton of 5-aminolevulinic acid (**3**). If (**3**) were the

Table 1. Incorporation of ^{14}C-labelled precursors into asukamycin by cultures of *Streptomyces nodosus* subsp. *asukaensis*.

Expt.	Precursor	Spec. act. (d.p.m./μmol) of precursor	Amount/μmol of precursor fed	Amount/μmol of (**2**) isolated	Spec. act. (d.p.m./μmol) of isolated (**2**)	Specific incorporation (%)
1	[2-^{14}C]Glycine	1.98×10^4	105	4.76	504	2.54
2	[2-^{14}C]Glycine	1.00×10^4	207	3.51	553	5.53
3	[2-^{14}C]Glycine	5.2×10^3	403	2.88	473	9.1
4	[2-^{14}C]Glycine	2.6×10^3	807	2.49	553	21.3
5	[1,4-^{14}C]Succinic acid	5.5×10^4	49	4.16	192	0.35
6	[1,4-^{14}C]Succinic acid	2.56×10^4	106	4.56	81	0.32
7	[1,4-^{14}C]Succinic acid	1.30×10^4	209	3.24	59	0.45
8	[1,4-^{14}C]Succinic acid	6.5×10^3	414	4.10	40	0.61
9	[5-^{14}C]-(**3**)·HCl	2.03×10^4	200[a]	5.57[a]	49	0.24
10	[5-^{14}C]-(**3**)·HCl	9.7×10^3	418[a]	4.87[a]	110	1.14
11	[5-^{14}C]-(**3**)·HCl	6.8×10^3	599[a]	2.12[a]	96	1.41
12	[5-^{14}C]-(**3**)·HCl	4.0×10^3	1010[a]	1.63[a]	87	2.18

[a] Two 100 ml cultures.



Scheme 1

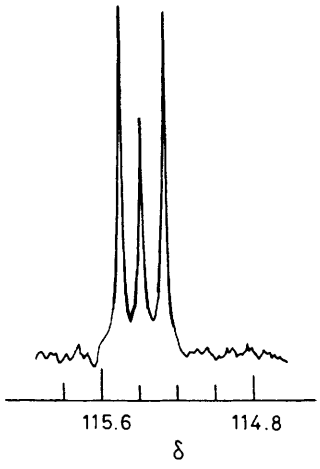


Figure 1. Signal for C-2″ in the ^{13}C n.m.r. spectrum of asukamycin derived from [2-^{13}C,^{15}N]glycine.

precursor of (**1**), C-1 of glycine should not be incorporated and carbon atoms 1, 3, 4, and 5 of (**1**) should arise from succinic acid. Although [1,4-^{14}C]succinic acid gave only low specific incorporations into (**2**) (Table 1, expts. 5—8), perhaps reflecting the Krebs cycle activity in this organism, other evidence supports the above idea. [1-^{13}C]Acetate labels C-1 and C-3 in the (**1**) moiety of (**2**) but not C-2, and the ^{13}C n.m.r. spectrum of (**2**) derived from [U-$^{13}C_3$]glycerol shows strong one-bond ^{13}C coupling satellites on the signals for C-1 and C-3 of the (**1**) moiety but only statistical coupling for C-2 (data not shown). The absence of more than statistical coupling in the signal for C-2″ rules out the alternative possibility that C-1 and C-2 of glycine are incorporated intact, since the glycerol would label glycine contiguously in both carbons *via* serine. The non-utilization of C-1 of glycine was confirmed directly by a comparison of the incorporations of [1-^{14}C]glycine (6.57×10^6 d.p.m., 56 μCi/μmol) and [2-^{14}C]glycine (7.35×10^6 d.p.m., 57 μCi/μmole), each fed to two flasks. The two experiments gave 4 628 and 45 666 total d.p.m. in asukamycin, corresponding to incorporations of 0.070% and 0.62%, respectively, *i.e.*, C-2 of glycine was utilized about 9 times better than C-1.

To evaluate further the role of (**3**), 5-amino-[5-^{14}C]levulinic acid was fed under a variety of conditions. Although (**3**) seemed to inhibit antibiotic synthesis and resulted in the

formation of copius amounts of dark pigments, moderately good specific incorporation values were consistently obtained with [5-^{14}C]-(**3**) (*e.g.*, Table 1, expts. 9—12). That these values were lower than the ones for glycine is not too surprising since apparently the bulk of the added (**3**) was diverted into other products. In addition, it may reflect low permeability of the cells for (**3**).

In view of the above results we suggest that the (**1**) moiety of (**2**) and other antibiotics is formed by a novel transformation of 5-aminolevulinic acid involving an intramolecular cyclization. A plausible mechanism for this conversion, involving a pyridoxal phosphate (PLP) enzyme, is shown in Scheme 1.

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