THE STRUCTURE OF STREPTONIGRONE, AND A COMMENT ON THE BIOSYNTHESIS OF THE STREPTONIGRIN ANTIBIOTICS

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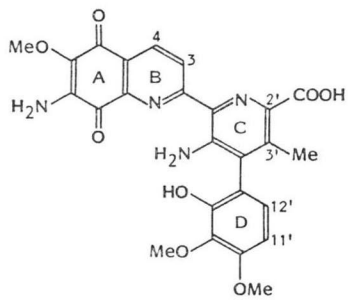
In a previous paper[1] we described the characterization and structure determination of the fujianmycins A and B, new benz[a]anthraquinone antibiotics isolated from an unidentified *Streptomyces* species collected in Fujian Province during a screening program conducted by the Institute of Antibiotics of the Chinese Academy of Medical Sciences. The same microorganism also produces the unusual antitumor and antimicrobial antibiotic streptonigrin (**1**)[2~5], together with a new, related metabolite which we have named streptonigrone. Comparison of the physical properties of these two metabolites defines the structure of streptonigrone as the 2-pyridone (**2**) corresponding to the 2-pyridinecarboxylic acid structure (**1**) of streptonigrin.

Fermentation of the *Streptomyces* species (IACAS isolate No. 114) and extraction of the metabolites from the culture filtrate (7.8 liters) adjusted to pH 6.5 was carried out as described previously[1]. The evaporated extract (929 mg) was partitioned between CH_2Cl_2 (100 ml) and cold aq $NaHCO_3$ (2%, 2×100 ml). The bicarbonate extracts were acidified to pH 6.0 with HCl (2 N), extracted with CH_2Cl_2 (3×200 ml), and the extracts washed with H_2O, dried, and evaporated. The residue (216 mg) was chromatographed on Sephadex LH-20 in MeOH - $CHCl_3$ (1:99), affording impure streptonigrin (120 mg) in the first, red fraction. Rechromatography in the same system gave streptonigrin (**1**) (100 mg), mp 269~272°C (ref 2; mp 275°C (dec)), MS (negative ion NH_3 chemical ionization) *m/z* 506.1443 (M, $C_{25}H_{22}N_4O_8$ requires 506.1437), 462.1569 (M−CO_2, $C_{24}H_{22}N_4O_6$ requires 462.1539), identified by UV, ^1H NMR and ^{13}C NMR spectra in comparison with literature data[2,5].

The bicarbonate-washed CH_2Cl_2 solution was evaporated (116 mg) and chromatographed on Sephadex LH-20 in MeOH - $CHCl_3$ (1:99). A greenish-yellow fraction (38 mg) eluted before fujianmycins A and B and was further purified by preparative TLC on silica gel in toluene - EtOAc (3:7). The resulting streptonigrone (**2**) (20 mg) formed rods from light petroleum - CH_2Cl_2, mp 268~269°C. MS (negative ion NH_3 chemical ionization) *m/z* 478.1485 (M, $C_{24}H_{22}N_4O_7$ requires 478.1488); UV λ_{max}^{MeOH} nm (ε) 425 (9,430), altered to 343 upon addition of HCl.

Although the electronic spectra of both streptonigrone and streptonigrin exhibit shifts indicative of amphoteric compounds on the addition of acid or strong base, streptonigrone is insoluble in aq sodium bicarbonate in contrast to the carboxylic acid streptonigrin. The molecular formula of streptonigrone, $C_{24}H_{22}N_4O_7$, differs from that of streptonigrin, $C_{25}H_{22}N_4O_8$, by the elements of CO. ^1H NMR spectra of the two compounds, listed in Table 1, show, however, that streptonigrone retains all the carbon-bound protons of streptonigrin together with their vicinal proton-proton couplings where present. In particular, the precise correspondence of chemical shifts of the three methoxyl groups, and of the higher field AB proton system, establishes that the A and D



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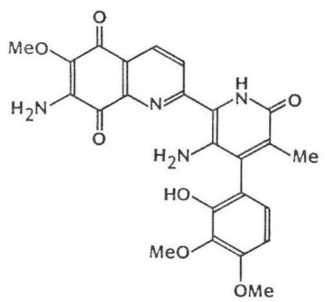

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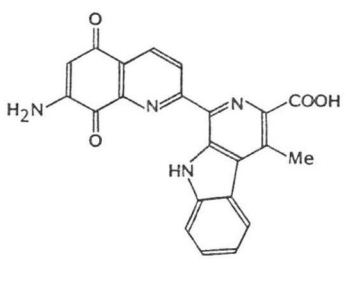

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Table 1. ^{1}H NMR data[a].

Assignment	Integral, multiplicity	Streptonigrin	Streptonigrone
3′-CH_3	3H, s	2.50	2.02
OCH_3	3H, s	3.96	3.95
OCH_3	3H, s	3.99	3.99
OCH_3	3H, s	4.10	4.07
5′-NH_2	2H, s	5.14	5.06
11′-H[b]	1H, d	6.68 (8)	6.65 (8.5)
12′-H[b]	1H, d	6.80 (8)	6.83 (8.5)
3-H	1H, d	8.47 (8)	8.31 (8)
4-H	1H, d	8.69 (8)	8.35 (8)

[a] For $CDCl_3$ solutions at 200 MHz, δ values in ppm from internal TMS, J in Hz in brackets.
[b] Assignment may be interchanged.

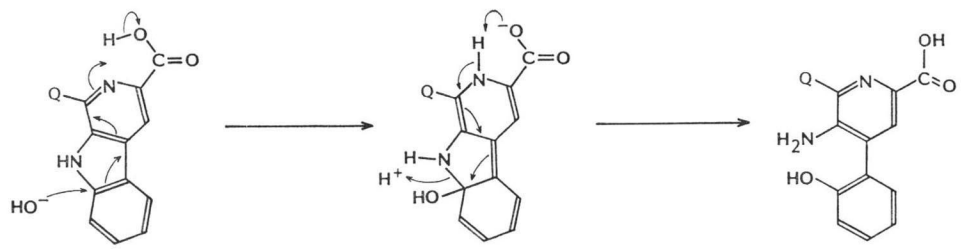
rings of streptonigrin (**1**) are unaltered in streptonigrone. The B ring of streptonigrin is also intact, as indicated by the lower field AB proton system, although the small shifts of these two protons indicate some change in their environment in streptonigrone. The greatest chemical shift difference, 0.48 ppm, between the two compounds occurs in the *C*-methyl groups attached to ring C. All these facts are uniquely accommodated by the formulation of streptonigrone as the 2-pyridone (**2**) corresponding to the 2-pyridinecarboxylic acid structure (**1**) of streptonigrin. The upfield shift to δ 2.02 of the 3′-methyl group on ring C of streptonigrone is in agreement with this structure (**2**), the corresponding resonance in 3-methyl-2-pyridone itself occurring at δ 2.05 (in DMSO)[6]. The strong light absorption of streptonigrone precluded optical rotation measurement, but its inherently dissymmetric skewed phenylpyridine chromophore probably has the same chirality as that of streptonigrin (suggested[7] to be *S*) in view of their co-occurrence in the present *Streptomyces* species.

Streptonigrone (**2**) showed no antimicrobial activity in disc assays at 50 μg/ml against strains of *Streptomyces aureofaciens, S. fragilis, Bacillus subtilis, Escherichia coli* and *Saccharomyces cerevisiae* on seeded agar plates. In contrast, streptonigrin (**1**) at this concentration inhibited all the test organisms, indicating the importance of the carboxyl function for such activity in agreement with earlier reports[5].

Streptonigrone (**2**) joins lavendamycin (**3**)[8,9] as the third structurally defined member of the streptonigrin group of streptomycete metabolites. Biosynthetic conversion of streptonigrin (**1**) or a precursor to streptonigrone (**2**) would involve only additional decarboxylation and oxidation steps, or alternatively covalent hydration[10] of the 1′,2′-bond of the pyridine ring followed by oxidative decarboxylation of the resulting α-hydroxyl acid.

The phenylpyridine ring system of streptonigrin, and presumably also of streptonigrone and lavendamycin, is derived biosynthetically from β-methyltryptophan[5,11~15]. GOULD and coworkers comment[5] that "such a cleavage reaction of an indole or a β-carboline, either chemically or biochemically, is unprecedented". They present[12,15] two possible mechanisms for the reaction, which involve alternative oxidations of an 8-hydroxy-β-carboline followed by ring cleavage and reduction of the resulting *o*-quinone. The overall process, however, is formally a hydrolysis, and we suggest it has parallels in the known ring opening of indoles bearing electron-withdrawing substituents[16,17]. It can be formulated (Scheme 1) as involving the covalent hydration[10] of a β-carboline system bearing two electron-withdrawing substituents (carboxyl and quinolinequinone) adjacent to the electronegative nitrogen of the pyridine ring. 1,6-Elimination from this hydrate of the original indole amino group, perhaps assisted by protonation, would then cleave the

Scheme 1. Possible biosynthetic origin of the phenylpyridine system.



center ring and generate the correctly substituted phenylpyridine system. Experiments to examine the feasibility of such a process are in hand.

Acknowledgments

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