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ON THE STRUCTURE OF ASPICULAMYCIN ITS IDENTITY WITH THE NUCLEOSIDE ANTIBIOTIC GOUGEROTIN

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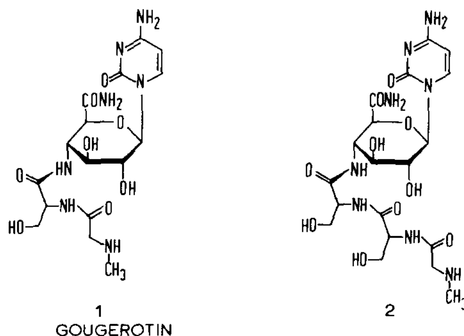
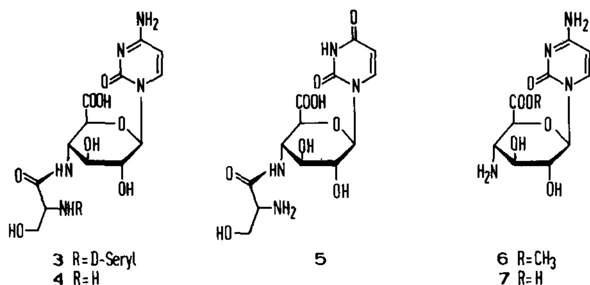
For aspiculamycin, a versatile anti-mycoplasma, acaridical and antibacterial antibiotic isolated recently from Streptomyces toyocaensis var. aspiculamyceticus[2, 3], structure 2 has been proposed on the basis of chemical and physicochemical evidence[4], hence being a close structural as well as biological[5, 6] analog to gougerotin (1) The structural proof presented is not entirely unambigous though, e.g. the molecular weight determined by osmometry was found to be 490[4] as compared to 530.5 for structure 2, and on mild acid treatment, 2 would be anticipated to preferentially cleave the sarcosine moiety[7] to form diseryl derivatives aside fully hydrolyzed products, rather than the reported[4] diseryl (3) and monoseryl compounds (5). The resumption of structural investigations on the antibiotic, however, was provoked by certain exploratory experiments directed towards its total synthesis, i e by the intriguing observation, that DCC-induced coupling of BOC-sarcosyl-D-seryl-D-serine[8] with 1-(methyl 4-amino-4-deoxy-ß-D-glucuronyl)cytosine (6), followed by successive treatment with methanolic ammonia and trifluoroacetic acid afforded a product which had the required UV-characteristics and analyzed correctly for a 2 1 ratio of serine and sarcosine[9], yet on t l c [10] proved to be clearly distinguishable from the natural substance[11]. As a result, we now present additional structural evidence for the antibiotic elaborated by S. toyocaensis var. aspiculamyceticus, that, in fact, proves it to be identical with gougerotin (1).



1 GOUGEROTIN   2



The assignment of structure 1 to the S. toyocaensis var. aspiculamyceticus derived product[11], i.e. its identity with gougerotin, rests on the following pieces of evidence.

First, its inhibitory activity on protein biosynthesis, as evaluated with the 70 S-promoted AcLeu-transfer from CACCA-LeuAc to puromycin (fragment reaction[13]) is identical with that observed for gougerotin[12].

Second, the product exhibited the same behaviour as gougerotin[12] on silica gel and cellulose coated t.l.c. plates in several solvent systems[10].

Third, the ratio of serine to sarcosine, as determined on an aminoacid analyzer[9] after acid hydrolysis of the product (6 N HCl, 3 h at 110°), was found to be 1 1.1 (± 0.1), i.e the same as that for gougerotin[12] (found 1 1.07).

Fourth, the 100 MHz nmr spectrum of the S. aspiculamyceticus derived product (Fig. 1) convincingly corresponds to what one has to expect for a 1-[4-deoxy-4-(sarcosyl-seryl)amino-ß-D-glucopyranuronamide)cytosine (1). Aside the three low field doublets for H-6, H-5 and the anomeric proton (H-1') a 1H-triplet is observed at 4.56 ppm, clearly attributable to the seryl-CH (H-2"), the other CH- and CH_2-protons fall into a complex pattern between 4.1 and 3.7 ppm, which on correlation to the N-CH_3-singlet of sarcosine (δ 2.80) clearly integrates for eight protons, i.e. the four sugar ring hydrogens H-2' - H-5' and the methylene protons of sarcosine (singlet at δ 3.99) and serine (doublet at δ 3.87). A sarcosyl-seryl-seryl derivative corresponding to structure 2 would have to give a 10H-integration for this pattern as well as a second triplet around 4.5 ppm.

In addition, the crystal form, melting behaviour and rotational value reported for aspiculamycin [needles decomposing at about 205°C, $[\alpha]_D^{20}$ + 54.9^{o} (c 1, water)] are in good agreement with the corresponding data on gougerotin, for which needles of m.p. 200-215° (dec)[5], 188-200° (dec)[6] and 211-217° (dec)[15] and $[\alpha]_D$-values of + 45° [5], + 53° [14] and + 57° [6] (c 0.8 - 1, water) have been observed. The same is true for the data on combustion analysis, since the C, H, N-values

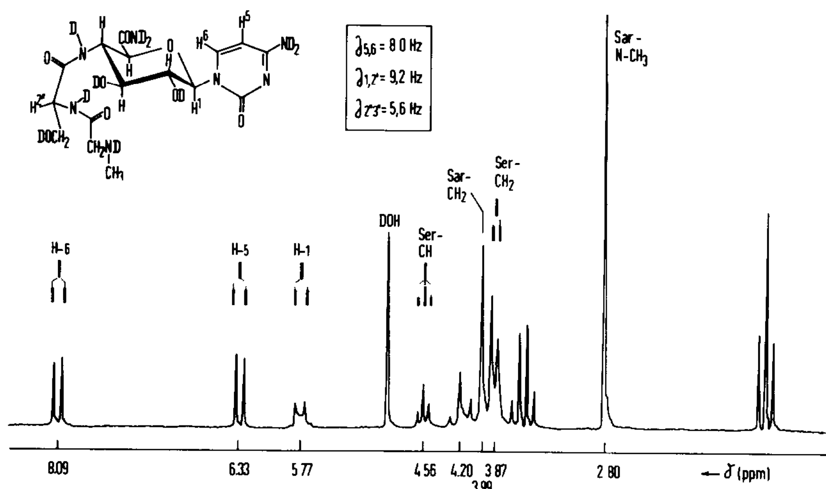


Fig. 1. 100 MHz-PFT spectrum in D_2O (100 pulse transients) of the S. aspiculamyceticus derived antibiotic, obtained from a 9 mg sample[11] that had repeatedly been reevaporated from 100 % D_2O to reduce the DOH peak obscuring of the triplet at 4.56 ppm, followed by addition of a trace of trifluoroacetic acid to shift the residual DOH signal. The sample contained 0.5 moles of ethanol.

for $C_{19}H_{25}N_7O_8$ (corresponding to structure 2) and $C_{16}H_{25}N_7O_8$ (gougerotin, 1) are too close as to allow a clear differentiation, i.e. between one and two serine moieties in the molecule. When comparing e.g. the analytical data found for the dipicrate of gougerotin (C, 37.40, H, 3.58, N, 19.94 %[5]) with those obtained for the S. aspiculamyceticus derived product (C, 37.65, H, 3.64, N, 19.80 %[4]), they are practically identical. Also, the osmometrically determined molecular weight of 490[4] corresponds well to a trihydrate of gougerotin[6] (497.4)

The supersedence of the aspiculamycin structure 2 by that of gougerotin (1) also requires that the diseryl-nucleoside 3, for which m.p. 214-216° (dec) and $[\alpha]_D^{20}$ + 16.8° (c 1, water) have been reported[4], is, in fact, the respective monoseryl-derivative, "seryl-C-substance" (4) of m.p. 230-235° (dec) and $[\alpha]_D^{27}$ + 57.3° (c 0.12, water)[14]. The discrepancy in rotational values will have to be cleared, yet an attractive explanation may lie in the low solubility of 4 in water, which allowed only c = 0.12 for the polarimetric solution[14], suggesting that at c = 1[4] dissolution in water was incomplete. Similarly, the crucial experiment leading to the proposition of structure 2, i.e. the acid hydrolysis of the 2,4-dinitrophenyl derivative of 3 to yield C-substance 7, DNP-serine and serine, will have to be clarified. Since experimental details were not given[4] an evaluation is not possible although an incomplete dinitrophenylation of 4, thus giving DNP-serine and serine on acid hydrolysis, does not appear to be an unlikely possibility.

In conclusion, it is worthy of noting that gougerotin which already has been isolated from three different streptomyces species obtained from soil samples of various parts of Japan[5,6,15], is equally elaborated by a streptomyces strain that also produces an as yet unidentified tetraene antibiotic and toyocamycin. Since the latter too has been isolated from various other streptomyces species[16], both, toyocamycin and gougerotin producing organisms appear to be of widespread occurence in Japan.

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(9) Determined on a Beckmann Unichrom Aminoacid Analyzer using an Aminex 6 column and a pH 3.24 buffer for elution at 30°; applied quantities of hydrolyzate corresponded to 75-100 nanomole of product; elution times were 55 min for serine and 60 min for sarcosine.

(10) Thin layer chromatography on Kieselgel F_{254} or cellulose F_{254} plastic sheets (Merck, Darmstadt) with n-butanol-acetic acid-water (5:2:3) or i-propanol-ammonia-water (7:1.2) as developers and detection by UV light. These systems proved quite effective in differentiating e.g. C-substance from its monoseryl and diseryl-derivative.

(11) We are indebted to Dr. M. Arai, Fermentation Research Laboratories, Sankyo Co., Tokyo, for providing a reference sample.

(12) A sample, commercially available from Calbiochem, USA, was used for comparisons.

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