BIOSYNTHESIS OF ANTHRACYCLINONES: ISOLATION OF A NEW EARLY CYCLIZATION PRODUCT AKLAVIKETONE

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Five metabolites were isolated from fermentations of a mutant strain S 383 of *Streptomyces galilaeus*. Components S 383-O and S 383-A were identified as known derivatives of anthraquinone and naphthacenequinone, respectively, previously isolated from cultures of other blocked mutants of *S. galilaeus* strains. Component S 383-X was identical with 7-deoxyaklavinone. Compound S 383-Y (aklaviketone) was found to be a new metabolite. Its chemical structure has been determined by physico-chemical methods including mass spectrometry and NMR spectral studies. The compound (7-dehydro-7-deoxy-7-oxoaklavinone) is most likely the first cyclization product along the metabolic chain possessing the tetracyclic carbon skeleton of anthracyclinones. A proposed pathway is discussed.

Recently, we confirmed that tricyclic compounds are involved in the biosynthetic pathway from the polyketide to aklavinone and related anthracyclinones[1~5]. However, only little is known about the intermediate steps prior to aklanonic acid and, on the other hand, about the mechanism of the formation of ring A leading to the characteristic tetrahydronaphthacenequinone skeleton of this class of antibiotics. During the course of our screening for new anthracyclines we investigated a number of blocked mutants that differ from the parent strains with respect to the metabolites accumulated. In this paper we describe the isolation and structure determination of a new metabolite that provided new information about the sequence of reactions from aklanonic acid to aklavinone. The compound, which is coproduced with several known metabolites, has been designated as aklaviketone.

Materials and Methods

Mass spectra were recorded on a Jeol JMS-D 100 spectrometer at 75 eV, temperature of direct inlet system 120~170°C. Exact mass measurements were performed using the peak matching technique (PFK as standard).

The ^{1}H NMR spectra were obtained at 100 MHz in $CDCl_3$ solution using a Tesla BS 497 spectrometer with FT adapter.

Mutant Strain and Fermentation Conditions

The producing mutant strain S 383 was obtained by treating spores of the parental strain *Streptomyces galilaeus* F 198 with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. The isolation of the mutant and fermentations were performed as described previously[5]. Normal fermentations of strain F 198 contained pyrromycinones and ε-pyrromycinone glycosides the major component being cinerubin A. Under the same conditions fermentations of mutant S 383 do not produce these compounds, *per se*, but accumulate a mixture of yellow, red and blue metabolites.

Isolation of the Metabolites

The crude mixture of S 383-components was obtained from the mycelium by extraction with acetone. The red extracts were combined and concentrated to about 1/3 of the volume. After ad-

dition of 1.5-volume portions of $CHCl_3$ and water the red $CHCl_3$ layer was separated, dried and evaporated to a small volume. Precipitation with petroleum ether afforded the pigment complex as a brown powder. The following components were detectable by TLC (Aluminium sheets Silica gel 60 F_{254} Merck, solvent system $CHCl_3$ - acetone (20 : 3)): S 383-A, red, Rf 0.14; S 383-P, blue, Rf 0.32; S 383-Y orange-yellow, color turning to red after two days, Rf 0.33; S 383-X, yellow, Rf 0.46; S 383-O, yellow, Rf 0.63; aklavinone standard, yellow, Rf 0.34.

Purification: The crude material was dissolved in a small amount of $CHCl_3$ - MeOH (90 : 10) and then subjected to column chromatography using Silica gel 60 (Merck). Elution was carried out with the solvent system $CHCl_3$ - MeOH (90 : 10) followed by the same system mixed, however, at a ratio of 80 : 20. The five components were eluted in the following series; yellow zone containing S 383-X and S 383-O, red zone containing S 383-Y, blue zone containing S 383-P and again a red zone with the component S 383-A. The compounds were isolated from the corresponding fractions by concentration and precipitation with petroleum ether. S 383-A was crystallized from benzene. The residue obtained from the first yellow zone was further purified by preparative TLC using analogous conditions as for analytical TLC. After extraction of the corresponding zones with MeOH, concentration, reextraction with a small amount of $CHCl_3$ and precipitation with petroleum ether compounds S 383-X and S 383-O were obtained as yellow substances. The new metabolite S 383-Y was obtained after precipitation with petroleum ether as a yellow powder or when concentrated to dryness as a red solid.

Identification of Known Metabolites

S 383-A

Red needles; mp 289~290°C; UV/VIS $\lambda_{max}^{CHCl_3}$ nm ($E_{1cm}^{1\%}$) 268 (1,660), 294 (357), 308 (240), 384 (96), 458 (sh, 284), 486 (449), 516 (sh, 403); IR ν_{max} (KBr) cm^{-1} 1722, 1675, 1630; MS *m/z* M^+ found 392.0898, calcd for $C_{22}H_{16}O_7$ 392.0896, 377.0673 $(M-CH_3)^+$, 361.0685 $(M-OCH_3)^+$, 333.0727 $(M-COOCH_3)^+$; triacetate *m/z* M^+ 518; found 518.1152, calcd for $C_{28}H_{22}O_{10}$ 518.1212; ^1H NMR, data see Table 1. The data are consistent with formula I (Fig. 1) which has been previously described by TOBE *et al.* as chemical structure of compound 665 B[6].

S 383-O

Yellow needles; mp not determined; UV/VIS ($CHCl_3$), maxima correspond with those reported in literature (KRÁLOVCOVÁ *et al.*[7] and TOBE *et al.*[6]); ^1H NMR Table 1. According to these properties compound S 383-O is identical with II (Fig. 1) first described by KRÁLOVCOVÁ *et al.*[7] and also found by TOBE *et al.*[6].

S 383-X

Yellow-orange needles; mp 223~225°C[8]; UV/VIS $\lambda_{max}^{CHCl_3}$ nm ($E_{1cm}^{1\%}$) 258 (685), 290 (270), 432 (314), 452 (sh, 272); MS *m/z* M^+ found 396.1218, calcd for $C_{22}H_{20}O_7$ 396.1209. Mass spectrum and IR spectrum were completely identical with those of 7-deoxyaklavinone (standard substance III, Fig. 1).

Properties of the New Compound S 383-Y

Yellow powder or red solid; mp not determined. The compound is unstable. UV/VIS $\lambda_{max}^{CHCl_3}$ nm 276, 290 (sh), 435; $\lambda_{max}^{CH_3OH}$ nm 243, 255 (sh), 287 (sh), 431. During the purification procedure by column chromatography and TLC or storage of the solution in MeOH at room temperature S 383-Y was partially converted to a red compound which is characterized by a maximum in the VIS spectrum at 532 nm (MeOH) and a Rf value similar to that of S 383-A. However, the degradation product is not identical with S 383-A.

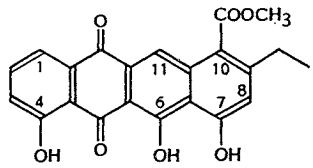
Further properties of S 383-Y: IR ν_{max} ($CHCl_3$) cm^{-1} 1630 (quinone C=O), 1655 (C=O), 1675

Table 1. ^{1}H NMR data of compounds aklaviketone (IV), S 383-A (I), and S 383-O (II) in $CDCl_3$ (100 MHz).

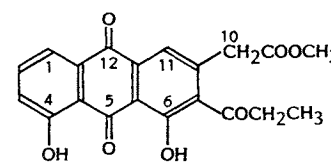
Assignment of protons[a]	Chemical shift, ppm (J, Hz)		
	IV	I	II
1-H	7.78 dd (A)	7.90 dd (A)	7.85 dd (A)
2-H	7.64 t (B)	7.71 t (B)	7.71 t (B)
3-H	7.33 dd (C) (J_{AB}=8, J_{AC}=1.6, J_{BC}=8)	7.32 dd (C) (J_{AB}=7.5, J_{AC}=1.7, J_{BC}=8)	7.34 dd (C) (J_{AB}=7.5, J_{AC}=1.6, J_{BC}=8)
11-H	7.76 s	8.23 s	7.73 s
4-OH	12.65 s	9.84 s	11.94 s
6-OH	13.94 br s	11.93 s	12.46 s
7-OH	—	15.51 br s	—
8-H	—	7.01 s	—
8-H_2	2.81, 3.40 (AB)	—	—
10-H	4.21 d (X) (J_{AB}=18, J_{AX}=1.8, J_{BX}=0)	—	—
10-H_2	—	—	3.81 s
CH_2CH$_3$	1.67 m	2.78 q	3.03 q
CH$_2$CH_3	1.09 t (J_{CH_2,CH_3}=7.5)	1.31 t (J_{CH_2,CH_3}=7.5)	1.21 t (J_{CH_2,CH_3}=7.5)
COOCH$_3$	3.74 s	4.07 s	3.71 s

[a] Numbering see Fig. 1.

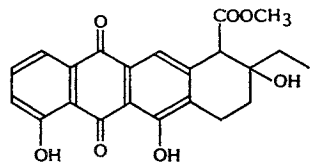
Fig. 1. Chemical structures of metabolites isolated from cultures of mutant S 383.
* Numbering corresponds to that used for anthracyclines.



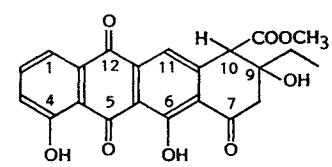
I



II*



III



IV (Aklaviketone)

(quinone C=O), 1738 (COOCH$_3$); MS m/z M$^+$ found 410.0998, calcd 410.1099 for $C_{22}H_{18}O_8$, 392.0895 (M−H_2O)$^+$, 361.0681 (M−H_2O−OCH_3)$^+$; ^{1}H NMR spectral data see Table 1.

Results and Discussion

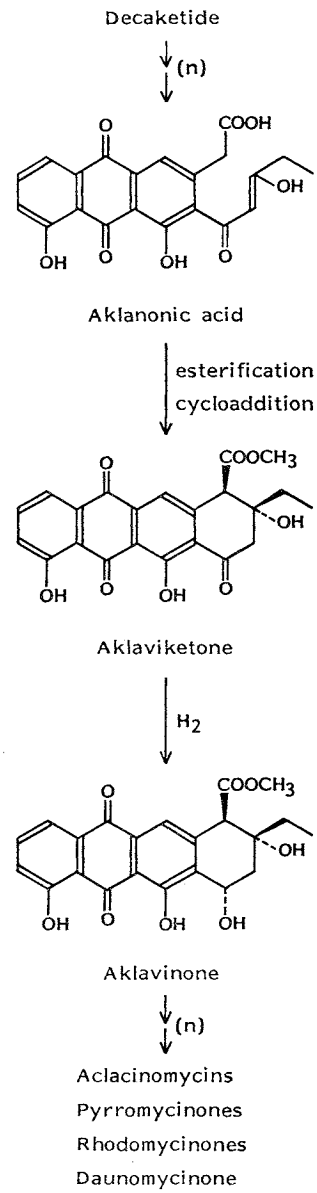
The metabolites of mutant S-383 were investigated to detect new intermediates in the biosynthetic

pathway from the decaketide precursor to anthracyclinones. The mutant strain S 383 was derived from a cinerubine-producing *S. galilaeus* wild strain. Out of the five metabolites isolated from the mycelium of the mutant strain three have been identified as follows: Considering the analytical data especially NMR spectra S 383-A is identical with compound 665 B (**I**, Fig. 1) previously described by TOBE *et al.*[6] although the melting point found for our compound differed markedly from that reported in literature. Component S 383-O (formula **II**, Fig. 1) is identical with an anthraquinone derivative first isolated and designated as "compound **XII**" by KRÁLOVCOVÁ *et al.*[7] and afterwards found by TOBE *et al.*[6] as a co-product with compound 665 B. Component S 383-X was found to be 7-deoxy-aklavinone (**III**, Fig. 1). The compounds were identified by comparison of their physico-chemical data with the properties described in literature or as in case of 7-deoxyaklavinone by direct comparison with authentic material isolated from an aclacinomycin-producing strain. Furthermore, the ^{1}H NMR spectra (Table 1) were in good agreement with the chemical structures described by TOBE *et al.* and KRÁLOVCOVÁ *et al.*

Component S 383-Y was found to be a new metabolite. Its chemical structure was determined by analysis of the UV/VIS, IR, ^{1}H NMR and mass spectra as follows: The UV/VIS spectrum of S 383-Y was similar to that of aklavinone indicating that the new compound is a derivative of 1,8-dihydroxyanthraquinone. This was also evident from the ^{1}H NMR spectrum which showed all characteristic signals of the anthraquinone part of aklavinone[6] caused by three neighbouring protons (ABC system, Table 1), one isolated aromatic proton (11-H singlet) and two chelated hydroxyls both, however, shifted downfield because of a keto group located at C-7 instead of OH and H in aklavinone. The 1,8-dihydroxyanthraquinone structure is also consistent with the observation of one chelated (1630 cm^{-1}) and one non-chelated quinone carbonyl (1675 cm^{-1}) in the IR spectrum.

With regard to the alicyclic ring the NMR spectrum of S 383-Y also shows strong similarities to that of aklavinone except of differences caused by different substituents at C-7. Thus, the characteristic signals of 10-H, CH_2 of C_2H_5, CH_3 of C_2H_5 and $COOCH_3$ (Table 1) indicate a complete alicyclic ring with a carbomethoxy at C-10 and

Fig. 2. Mode of the formation of the tetracyclic skeleton in the biosynthesis of anthracyclinones.



OH/C_2H_5 at C-9. As mentioned before, the substituent at C-7 in the molecule of S 383-Y was found to be a keto group. This was evident from the following facts:

1) The AB system assigned to the two 8-H protons (Table 1) is shifted downfield and shows only simple coupling pattern indicating that, contrary to aklavinone, C-7 carries no proton.

2) The IR spectrum exhibits besides the quinone bands and the band of an ester carbonyl (1738 cm^{-1}) an additional carbonyl absorption band at 1655 cm^{-1}. The absorption at 1655 cm^{-1} indicates strong chelation with phenolic hydroxyl and is in good agreement with the location of the CO group at C-7.

3) The presence of CO at C-7 explains the large geminal coupling (J=18 Hz) between the two protons at C-8. Further information was provided by the observation of a long range coupling of one of these protons at C-8 with the proton at C-10 ("W" constellation) that indicates equatorial arrangement of the C-10 proton and gives additional confirmation for the closed alicyclic ring A. All mentioned facts allowed to formulate **IV** as chemical structure of S 383-Y.

Recently, we have shown in a series of biotransformation experiments that the biosynthesis of anthracyclinones proceeds *via* a tricyclic intermediate which we have given the name aklanonic acid. The isolation of compound S 383-Y provides evidence that ring closure to the characteristic tetrahydronaphthacenequinone skeleton of anthracyclinones is commonly achieved by cycloaddition mechanism as shown in Fig. 2. This reaction is then followed by reduction to give aklavinone, the first complete anthracyclinone which is the precursor for aclacinomycins and other anthracyclines like pyrromycins, rhodomycins, isorhodomycins or daunomycin and related antibiotics. Since compound S 383-Y is an essential intermediate between aklanonic acid and aklavinone we have designated this compound as aklaviketone according to its characteristic keto group located in ring A. Preliminary biotransformation experiments with aklaviketone support the proposed scheme in Fig. 2.

Aklaviketone is not the only compound possessing this characteristic structure of ring A. McGUIRE and co-workers[10] have described the isolation of maggiemycin from a natural variant of a daunomycin-producing *Streptomyces* strain which is presumably formed from aklaviketone by subsequent hydroxylation of the free position of ring B[11].

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