Communications to the editor

ANSATRIENIN A_2 AND A_3: MINOR COMPONENTS OF THE ANSAMYCIN COMPLEX PRODUCED BY *STREPTOMYCES COLLINUS*

Sir:

The ansatrienins produced by *Streptomyces collinus* ssp. *collinus* (Lindenbein) strain Tü 1982 represent a new type of benzoquinoid ansamycin antibiotic[1~3]. Ansatrienin B, the hydroquinone of A, seems to be identical with mycotrienin, described in 1967[4]. Recently the mycotrienins I and II were isolated from *Streptomyces rishiriensis*[5~7], the given structures are the same previously elucidated for ansatrienin A (**1**) and B[1,2] except the configuration of alanine. In this communication we report the isolation and structural data of two new ansatrienins.

The ansatrienin complex was extracted from the mycelium of *Streptomyces collinus* with acetone[1]. The crude product is a mixture of different benzoquinone (A) and hydroquinone (B) components. The ansatrienin complex was oxidized by silver oxide in acetone or $FeCl_3$ solution in ethyl acetate. The A-components were easily separated on silica gel (PTLC) with chloroform - methanol (96: 4). Two fractions were collected, yielding **1** (27 mg/liter culture broth) and a mixture of minor components. The latter were then applied to a silica gel column (HPLC) and eluted with a mixture of *n*-hexane - ether - acetone (5: 4: 1). Ansatrienins A_2 (**2**) and A_3 (**3**) were obtained in equal amounts (4 mg/liter culture broth) as yellow amorphous powder. The Rf values are given in Table 1.

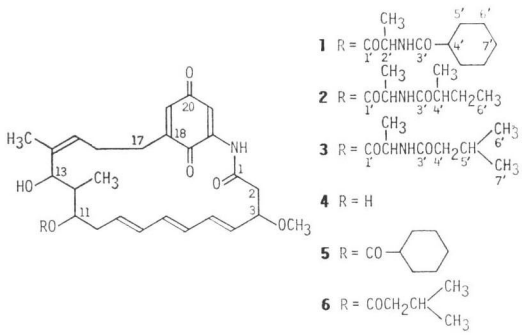
Table 1. Rf values (TLC, silica gel) of the ansatrienins.

Solvent system	A (**1**)	A_2 (**2**)	A_3 (**3**)
Toluene - EtOAc (3: 2)	0.37	0.28	0.23
Ether	0.27	0.25	0.22
$CHCl_3$ - MeOH (96: 4)	0.47	0.45	0.41

The new ansatrienins are isomers and differ only slightly in their physico-chemical properties (Table 2). The IR and UV spectra are very similar to those of ansatrienin A (**1**) indicating the same chromophoric systems[1]. **2** and **3** have a smaller molecular formula than **1**, differing by C_2H_2. The alteration is located in the *N*-acyl alanine side chain of **1**. This derives from the following data: 1) EI-MS (high resolution) reveals the molecular ion at *m/z* 610, which loses $C_8H_{15}NO_3$ (173) giving the fragment peak at *m/z* 437 ($C_{26}H_{31}NO_5$). This key fragment also was found in the mass spectrum of **1**, there, however, formed by loss of *N*-cyclohexylcarbonyl alanine $C_{10}H_{17}NO_3$ (199) from M^+[1]. 2) Reduction of

Table 2. Physico-chemical properties of ansatrienins A_2 and A_3.

	A_2 (**2**)	A_3 (**3**)
Melting point	115°C (decomp.)	117°C (decomp.)
$[\alpha]_D^{20}$	+115.7° (*c* 0.75, $CHCl_3$)	+119.4° (*c* 0.65, $CHCl_3$)
Elemental analysis (%) Found	C 67.05, H 7.74, N 4.68	C 66.42, H 7.38, N 4.40
Calcd.	C 66.87, H 7.59, N 4.59	C 66.87, H 7.59, N 4.59
Molecular formula	$C_{34}H_{46}N_2O_8$	$C_{34}H_{46}N_2O_8$
EI-MS: M^+ (%)	*m/z* 610.3254 (1%)	*m/z* 610.3254 (4%)
UV (MeOH): λ_{max} (ε)	387 (1800), 279 (37100), 271 (46200), 264 sh, 230 nm (24400)	the same
UV (MeOH/NaOH): λ_{max} (ε)	481 (1900), 278 (43500), 269 (48900), 261 nm (43600)	the same
IR (KBr)	1730, 1710, 1662 sh, 1650, 1630 sh, 1608 cm^{-1}	the same
CD (MeOH): λ_{max} ($[\theta]^{21} \cdot 10^{-4}$)	284 (−15.0), 278 sh (−7.4), 264 (+13.0), 258 nm (+16.0)	284 (−11.6), 278 sh (−5.6), 264 sh (+10.8), 258 nm (+12.8)



2 and **3** with $LiAlH_4$ in tetrahydrofuran (1 hour/ −28°C) afforded ansatrienol A (**4**) which is completely identical with the compound isolated from **1**[2,3]. 3) Hydrolysis of **2** and **3** in aqueous alkaline solution[8] gave 2-methylbutyric acid and isovaleric acid, respectively, which were separated on Dowex 50WX8 and identified by their retention time (GC, 60/80 Carbopack C - 0.3% Carbowax 20 M - 0.1% H_3PO_4, 150°C) in comparison with authentic samples.

The ^1H NMR spectrum of **2** indicates the presence of alanine by signals at δ 1.45/4.40 for 2′-CH_3/2′-H and at δ 5.89 for NH. When compared with the spectrum of **1** the signals for the cyclohexyl protons are replaced by those for a 2-methylbutyryl residue: δ 0.92 (t, 3H), 1.13 (d, 3H), 1.65 (m, 2H) and 2.22 (m, 1H). The corresponding signals for **3** (Fig. 1) appeared at δ 0.95 (d, 6H) and 2.06 (m, 3H) suitable for isovaleryl. The remaining ^1H NMR signals derive from the ansa ring protons, the deviation from the δ values given for **1**[2] is less than 0.1 ppm. The ^{13}C NMR data are in agreement[9] with the assigned structure elements (Table 3); the chemical shifts of the ansa ring carbons are in accordance with the data given for **1**[1].

Table 3. ^{13}C NMR data of the *N*-acyl alanine side chain in $CDCl_3$.

Assignment	1	2	3
C-1′	172.7 (s)	172.6 (s)	172.6 (s)
C-2′	48.7 (d)	48.6 (d)	48.7 (d)
2′-CH_3	17.3 (q)	17.5 (q)	17.4 (q)
C-3′	176.5 (s)	176.7 (s)	172.9 (s)
C-4′	44.7 (d)	42.5 (d)	45.3 (t)
4′-CH_3	—	17.1 (q)	—
C-5′	29.6 (t)	27.2 (t)	26.2 (d)
C-6′	25.6 (t)	11.8 (q)	22.4 (q)
C-7′	25.6 (t)	—	22.4 (q)

50.5 MHz, δ values in ppm relative to internal TMS.

The ansatrienins are active against fungi. The weak activity of the A-components against Gram-positive bacteria is antagonized by cysteine[1]. **1** and **2** show comparable activity while **3** is considerably more active against fungi (Table 4). To investigate the role of L-alanine with respect to biological activity, ansatrienol A (**4**) was esterified directly with cyclohexylcarboxylic acid in acetone (DCC/dimethylaminopyridine, −20°C,

Fig. 1. ^1H NMR spectrum of ansatrienin A_3 (**3**) in $CDCl_3$ at 200 MHz.

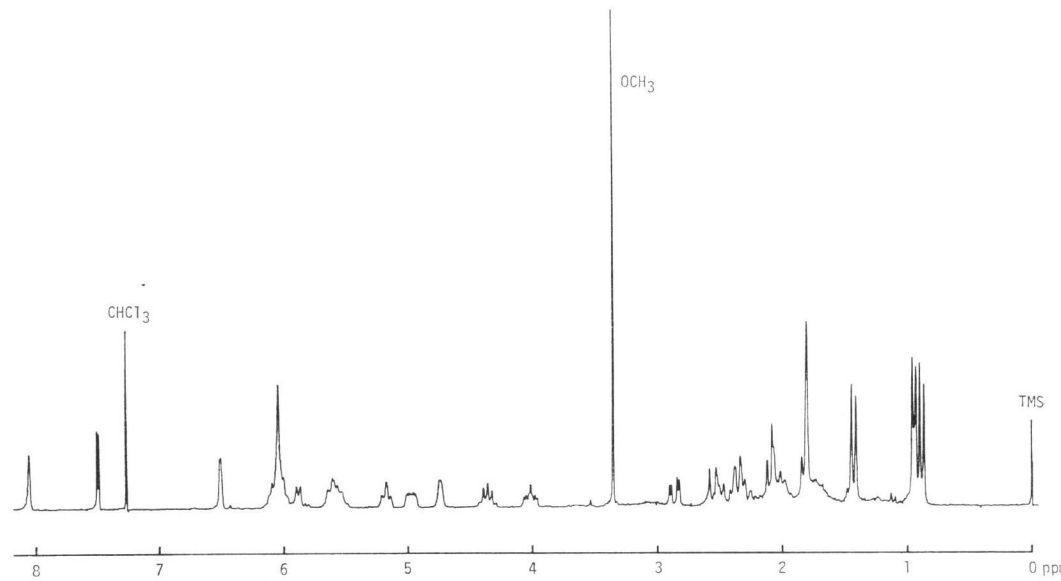


Table 4. Antifungal activity of the ansatrienins (agar diffusion method, 6 mm paper disk, inhibitory diameter in mm).

Test organism	1 (1 mg/ml)	2 (1 mg/ml)	3 (0.5 mg/ml)
Botrytis cinerea	32	33	32
Mucor hiemalis 179/180	10	8	11.5
Mucor miehei	15	12.5	17
Mucor mucedo	15	—	15
Mucor parvisporus	20	17	23
Trametes zonata	28	29	33
Saprolegnia asterophora	23	24	29
Geotrichum candidum	—	8	12

90 minutes) and isovaleric anhydride in pyridine (20°C, 24 hours), respectively. We obtained 11-cyclohexylcarbonyl-ansatrienol A (**5**, 66%) and 11-isovaleryl-ansatrienol A (**6**, 54%) together with the corresponding 11,13-diacyl derivatives[8]. When compared with **4**, the monoesters show a new IR ester band at 1728 cm^{-1}. The ^1H NMR spectra ($CDCl_3$, 200 MHz) give evidence for one ester side chain in **5** and **6**. The paramagnetic shift of 11-H (δ 3.80 in **4** to δ 4.89 in **5** and δ 4.94 in **6**, respectively) confirms that the ester is attached at the corresponding hydroxyl group (11-OH). 13-H shows a small upfield shift (δ 4.84~4.40). **5** and **6** are inactive against the tested fungi and bacteria indicating that L-alanine is an essential part of the ansatrienins. We assume that the structure-activity relationship of the ansatrienins is quite different from the maytansinoids.[10]

Acknowledgement

We thank the Deutsche Forschungsgemeinschaft for financial support.

GÜNTER LAZAR
HANS ZÄHNER
Institut für Biologie II, Lehrstuhl Mikrobiologie I, Universität Tübingen
Auf der Morgenstelle 28, D-7400 Tübingen, F.R.G
MANFRED DAMBERG
AXEL ZEECK*
Institut für Organische Chemie, Universität Göttingen, Tammannstr. 2, D-3400 Göttingen, F.R.G.

(Received September 27, 1982)

* To whom communications should be addressed.

References

1) WEBER, W.; H. ZÄHNER, M. DAMBERG, P. RUSS & A. ZEECK: Stoffwechselprodukte von Mikroorganismen. 201. Ansatrienin A und B, fungistatische Antibiotica aus *Streptomyces collinus*. Zbl. Bakt. Hyg., I. Abt. Orig. C 2: 122~139, 1981
2) DAMBERG, M.; P. RUSS & A. ZEECK: Die Konstitution der fungistatischen Ansamycin-Antibiotica Ansatrienin A und B. Tetrahedron Lett. 23: 59~62, 1982
3) DAMBERG, M.: Isolierung, Strukturaufklärung und Derivatisierung neuer Ansamycin-Antibiotica aus Streptomyceten. Ansatrienine und Naphtomycin C. Thesis Univ. Göttingen, 1982
4) CORONELLI, C.; R. C. PASQUALUCCI, J. E. THIEMANN & G. TAMONI: Mycotrienin, a new polyene antibiotic isolated form *Streptomyces*. J. Antibiotics 20: 329~333, 1967
5) SUGITA, M.; K. FURIHATA, H. SETO, N. ŌTAKE & T. SASAKI: The structures of mycotrienins I and II, a novel class of ansamycin antibiotic. Agric. Biol. Chem. 46: 1111~1113, 1982
6) SUGITA, M.; Y. NATORI, T. SASAKI, K. FURIHATA, A. SHIMAZU, H. SETO & N. ŌTAKE: Studies on mycotrienin antibiotics, a novel class of ansamycins. I. Taxonomy, fermentation, isolation and properties of mycotrienins I and II. J. Antibiotics 35: 1460~1466, 1982
7) SUGITA, M.; T. SASAKI, K. FURIHATA, H. SETO & N. ŌTAKE: Studies on mycotrienin antibiotics, a novel class of ansamycins. II. Structure elucidation and biosynthesis of mycotrienins I and II. J. Antibiotics 35: 1467~1474, 1982
8) ASAI, M.; E. MIZUTA, M. IZAWA, K. HAIBARA & T. KISHI: Isolation, chemical characterization and structure of ansamitocin, a new antitumor ansamycin antibiotic. Tetrahedron 35: 1079~1085, 1979
9) COUPERUS, P. A.; A. D. CLAQUE & J. P. VAN DONGEN: Carbon-13 chemical shifts of some model carboxylic acids and esters. Org. Mag. Reson. 11: 590~597, 1978
10) KOMODA, Y. & T. KISHI: Maytansinoids. Medicinal Chemistry. Vol. 16, Anticancer Agents Based on Natural Product Models. *Ed.* by. J. M. CASSADY & J. D. DOUROS, pp. 353~390, Academic Press, New York, 1980