(*E*)-4-Oxonon-2-enoic Acid, an Antibiotically Active Fatty Acid Produced by *Streptomyces olivaceus* Tü 4018†

CHRISTOPH PFEFFERLE, CHRISTOPH KEMPTER[a], JÖRG W. METZGER[a] and HANS-PETER FIEDLER*

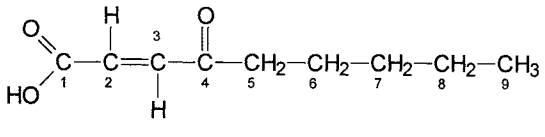
Biologisches Institut, Universität Tübingen, Auf der Morgenstelle 28, D-72076 Tübingen, Germany
[a]Institut für Organische Chemie, Universität Tübingen, Auf der Morgenstelle 18, D-72076 Tübingen, Germany

(Received for publication May 7, 1996)

Streptomyces olivaceus Tü 4018 was included in our screening programme for detection and isolation of new secondary metabolites. Extracts of actinomycete strains were analysed by HPLC coupled with diode array multiwavelength monitoring (HPLC-DAD) and a UV-visible absorbance spectral library database[2]. The kanchanamycins, a complex of new 36-membered polyol-type macrolide antibiotics[1,3], the 42-membered macrolactones desertomycin A[4] and oasomycin A[5], and tryptophan-dehydrobutyrine diketopiperazine[6] as well as the isoflavone daidzein were isolated from strain Tü 4018. All metabolites were produced during fermentation in a complex medium consisting of 2% mannitol and 2% soybean meal.

In addition to these compounds, *S. olivaceus* Tü 4018 produced under modified cultivation conditions a substance which was detected in the mycelium extract by HPLC-DAD analysis (HP 1090M liquid chromatograph equipped with a built-in diode array detector and HPLC^{3D}-ChemStation, Hewlett-Packard). This compound was not identified by spectral matching techniques using our HPLC-DAD database. HPLC-ESI-MS analysis (ABI 140A HPLC system, Applied Biosystems; API III triple-quadrupol mass spectrometer equipped with a nebulizer-assisted electrospray ion source, Sciex) determined a molecular mass of 170.1, and search in commercially available databases lead to further evidence for the novelty as a natural product. Isolation and structure elucidation resulted in determination of the compound as (*E*)-4-oxonon-2-enoic acid (Fig. 1) that has not previously been described as a natural product, but has been made synthetically in the course of a new method for preparation of (*E*)-3-acylprop-2-enoic acids[7]. Fatty acids are produced by actinomycetes mostly as constituents of complex compounds, such as the lipopeptide antibiotics amphomycin[8] and A21978 C antibiotic complex[9]. Single fatty acid derivatives however, such as cerulenin[10], or condensed derivatives, such as myriocin[11], were mainly isolated from fungi.

Fig. 1. Structure of (*E*)-4-oxonon-2-enoic acid.



Strain *S. olivaceus* Tü 4018 was cultivated in 500-ml Erlenmeyer flasks with one baffle on a rotary shaker at 120 rpm and 27°C containing 100 ml of medium which consisted of mannitol 2% and soybean meal 2% in tap water (pH 7.5). A 10-litre stirred tank fermenter (Biostat E) was inoculated with 5% (v/v) of a 24-hours preculture, and grown at 27°C, aeration rate of 0.5 v/v/m and agitation of 300 rpm. Addition of 5 mM cobalt sulphate 36 hours after inoculation resulted in production of (*E*)-4-oxonon-2-enoic acid at about 60 hours. The production reached a maximum after 96 hours at a concentration of 3.1 mg/litre. Production of (*E*)-4-oxonon-2-enoic acid was not observed at all during previous fermentations that were carried out without addition of cobalt sulphate.

(*E*)-4-oxonon-2-enoic acid was isolated from the mycelium by extraction twice with ethanol. The combined extracts were concentrated *in vacuo* to the aqueous residue that contained 31 mg of the fatty acid. The concentrate was purified by column chromatography using Amberlite XAD-16. (*E*)-4-oxonon-2-enoic acid was desorbed from the resin by MeOH–H_2O (40+60), concentrated *in vacuo*, and extracted at pH 4 (1 N HCl)

Table 1. Physico-chemical properties of (*E*)-4-oxonon-2-enoic acid.

Appearance	White powder
EI-MS (*m/z*)	
Found:	171.101 $[M+H]^+$
Calcd:	171.102
Molecular formula	$C_9H_{14}O_3$
UV λ_{max}^{MeOH} nm	242
IR (KBr) cm^{-1}	3406, 3070, 2956, 2931, 1679, 1660, 1623

Table 2. ^1H and ^{13}C NMR chemical shifts of (*E*)-4-oxonon-2-enoic acid.

Carbon No.	^{13}C chemical shifts ppm	^1H chemical shifts ppm
C1	169	—
C2	141	7.1 (d, J_{HH}=16.2 Hz)
C3	129	6.64 (d, J_{HH}=16.2 Hz)
C4	199	—
C5	42	2.6 (t)
C6	31	1.6 (m)
C7	23	1.3 (m)
C8	22	1.3 (m)
C9	14	0.9 (t)

Spectra were recorded in $CDCl_3$ solution; coupling constants are given in Hz.

† Art. No. 8 on biosynthetic capacities of actinomycetes. Art No. 7: See ref. 1.

Table 3. Antimicrobial spectrum of (*E*)-4-oxonon-2-enoic acid, determined by the agar plate diffusion test, inhibition zones in mm.

Test organism	(*E*)-4-oxonon-2-enoic acid conc.		
	3 mg/ml	1 mg/ml	0.3 mg/ml
Bacillus subtilis ATCC 6051[a]	21	12	—
Bacillus subtilis ATCC 6051[b]	13	(8)	—
Bacillus brevis ATCC 9999	20	13	—
Micrococcus luteus ATCC 381	18 (21)	15 (17)	(10)
Staphylococcus aureus ATCC 11632	22	15	10
Arthrobacter aurescens ATCC 13344[a]	—	—	—
Arthrobacter aurescens ATCC 13344[b]	28	18	—
Streptomyces viridochromogenes Tü 57	—	—	—
Agrobacterium tumefaciens ATCC 15955[a]	9 (12)	—	—
Agrobacterium tumefaciens ATCC 15955[b]	16	8	—
Escherichia coli K 12[a]	13	(10)	—
Escherichia coli K 12[b]	14	—	—
Proteus mirabilis ATCC 29906[a]	(18)	(10)	—
Proteus mirabilis ATCC 29906[b]	(14)	(8)	—
Pseudomonas fluorescens ATCC 13525[a]	—	—	—
Candida albicans ATCC 10231[a,b]	—	—	—
Rhodotorula rubra Tü 8093[a,b]	—	—	—
Saccharomyces cerevisiae Tü 125[a]	—	—	—
Botrytis cinerea Tü 157[c]	—	—	—
Mucor mieheii Tü 284	—	—	—
Paecilomyces variotii Tü 137[a]	15	(9)	—
Paecilomyces variotii Tü 137[b]	16	(9)	—
Penicillium notatum Tü 136[a,b]	—	—	—

[a] Complex medium.
[b] Chemically defined medium (per litre): glucose 5 g, tri-Na-citrate·$2H_2O$ 0.5 g, KH_2PO_4 3 g, K_2HPO_4 7 g, $MgSO_4 \cdot 7H_2O$ 0.1 g, $(NH_4)_2SO_4$ 1 g, Bacto agar 15 g.
[c] Giant colony.
( ) Diffuse inhibition zone.

with ethyl acetate. The organic layer was concentrated to dryness. Separation of the raw product was carried out by size-exclusion chromatography on a Sephadex LH-20 column using MeOH as eluent. Pure (*E*)-4-oxonon-2-enoic acid was obtained by preparative reversed-phase HPLC using Nucleosil-100 C-18 (particle size 10 μm, i.d. 16 mm × 250 mm stainless steel column) and linear gradient elution with 0.5% aqueous acetic acid - MeOH, starting from 40% MeOH to 80% MeOH within 10 minutes and a flow rate of 20 ml/minute. The compound was obtained after lyophilisation as a white amorphous powder in an amount of 16 mg. The physicochemical properties are summarised in Table 1.

By measuring a high resolution EI-MS spectrum (MAT 711, Finnigan), the protonated molecular ion $[M+H]^+$ of (*E*)-4-oxonon-2-enoic acid was determined as 171.101 corresponding to the molecular formula $C_9H_{15}O_3$. The structure elucidation was done using homonuclear (TOCSY[12]) and heteronuclear (HSQC[13], HMBC[14]) 2D NMR spectra. The J_{HH} coupling constant of 16.2 Hz indicated *E*-configuration at the double bond C2=C3.

The antimicrobial activity of (*E*)-4-oxonon-2-enoic acid was tested by the agar plate diffusion assay and is shown in Table 3. An antibacterial activity against various Gram-positive and Gram-negative bacteria was observed, especially against *Staphylococcus aureus* ATCC 11632 at a concentration of 0.3 mg/ml, but not against yeasts and other fungi with the exception of *Paecilomyces variotii*.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 323) and the Fonds der Chemischen Industrie. The authors wish to thank Mr. A. KULIK and Mr. M. NEGA for technical assistance.

References

1) STEPHAN, H.; C. KEMPTER, J. W. METZGER, G. JUNG, O. POTTERAT, C. PFEFFERLE & H.-P. FIEDLER: Kanchanamycins, new polyol macrolide antibiotics produced by *Streptomyces olivaceus* Tü 4018. II. Structure elucidation. J. Antibiotics 49: 765～769, 1996
2) FIEDLER, H.-P.: Biosynthetic capacities of actinomycetes. 1. Screening for secondary metabolites by HPLC and UV-visible absorbance spectral libraries. Nat. Prod. Lett. 2: 119～128, 1993
3) FIEDLER, H.-P.; M. NEGA, C. PFEFFERLE, I. GROTH, C. KEMPTER, H. STEPHAN & J. W. METZGER: Kanchanamycins, new polyol macrolide antibiotics produced by *Streptomyces olivaceus* Tü 4018. I. Taxonomy, fermentation, isolation and biological activities. J. Antibiotics 49: 758～764, 1996

4) URI, J. V.: Desertomycin: a potentially interesting antibiotic. Acta Microbiol. Hung. 33: 271~283, 1986
5) GRABLEY, S.; G. KRETSCHMER, M. MAYER, S. PHILLIPS, R. THIERICKE, J. WINK & A. ZEECK: Oasomycins, new macrolactones of the desertomycin family. Liebigs Ann. Chem. 1993: 573~579
6) KAKINUMA, K. & K. L. RINEHART Jr.: Tryptophan-dehydrobutyrine diketopiperazine, a metabolite of *Streptomyces spectabilis*. J. Antibiotics 27: 733~737, 1974
7) OBRECHT, D. & B. WEISS: A new method for the preparation of (*E*)-3-acylprop-2-enoic acids. Helv. Chim. Acta 72: 117~122, 1989
8) HEINEMANN, B.; M. A. KAPLAN, R. D. MUIR & I. R. HOOPER: Amphomycin, a new antibiotic. Antibiot. Chemother. 3: 1239~1242, 1953
9) ZMIJEWSKI, Jr., M. J.; B. BRIGGS & J. OCCOLOWITZ: Role of branched chain fatty acid precursors in regulating factor profile in the biosynthesis of A21978 C complex. J. Antibiotics 39: 1483~1485, 1986
10) SANO, Y.; S. NOMURA, Y. KAMIO, S. ŌMURA & T. HATA: Studies on cerulenin, III. Isolation and physico-chemical properties of cerulenin. J. Antibiotics 30: 344~348, 1967
11) KLUEMPFEL, D.; J. BAGLI, H. BAKER, M.-P. CHAREST, A. KUDELSKI, S. N. SEHGAL & C. VEZINA: Myriocin, a new antifungal antibiotic from *Myriococcum albomyces*. J. Antibiotics 25: 109~115, 1972
12) BAX, A. & D. G. DAVIS: MLEV-17 based two-dimensional homonuclear magnetization transfer spectroscopy. J. Magn. Reson. 65: 355~360, 1985
13) BODENHAUSEN, G. & D. J. REUBEN: Natural abundance nitrogen-15 NMR by enhanced heteronuclear spectroscopy. Chem. Phys. Lett. 69: 185~189, 1980
14) BAX, A. & A. M. SUMMERS: ^{1}H and ^{13}C assignment for sensitivity-enhanced detection of heteronuclear multiple-bond connectivity by 2D multiple quantum NMR. J. Am. Chem. Soc. 108: 2093~2094, 1986