Sesquicillin, an Inhibitor of Glucocorticoid Mediated Signal Transduction

BIBORKA ENGEL, GERHARD ERKEL*,
TIMM ANKE and OLOV STERNER†

Lehrbereich Biotechnologie der Universität Kaiserslautern, Paul Ehrlich-Strasse 23, D-67663 Kaiserslautern, F.R.G.
†Division of Organic Chemistry 2, University of Lund, P.O.B. 124, S-221 00 Lund, Sweden

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Glucocorticoids have been used for decades as anti-inflammatory, anti-allergic and immunosuppressive agents. Inhibition of the inflammatory response is mediated *via* transactivation of glucocorticoid responsive genes and transrepression of genes responsive to the transcription factors AP1 or NF-κB, which regulate the expression of pro-inflammatory cytokines and other genes involved in inflammatory processes[1]. However, the immunosuppressive mechanism of action of glucocortocoids is poorly understood. Glucocorticoid antagonists would provide a good tool to carry out such studies.

In the course of a screening for fungal inibitors of the glucocorticoid mediated signal transduction, COS-7 cells were cotransfected with a reporter gene plasmid carrying the reporter gene secreted alkaline phosphatase (SEAP) under the control of 3 copies of the glucocorticoid response element (GRE) together with a human glucocorticoid receptor-α expression vector. This led to the isolation of sesquicillin from an *Acremonium* sp. Sesquicillin has previously been reported in patents[2,3], and is described to have antihypertensive, bronchospasmolytic, anti-inflammatory and laxative activities[2]. However, these effects have not been attributed to effects on glucocorticoids, the NMR spectroscopic data of sesquicillin have not been reported, and the elucidation of its structure has not been described.

Experimental

Fermentation and Isolation

For maintenance on slant agars and submerged cultivation, *Acremonium* sp., strain 132-94, publicly available from the department of Biotechnology, University of Kaiserslautern, was grown in YMG medium composed of: yeast extract 0.4%, malt extract 1%, glucose 0.4%, pH 5.5 and agar 1.5% for solid media. The strain is deposited in the culture collection of the LB Biotechnologie, Universität Kaiserslautern. Fermentations were carried out in a Biolafitte C-6 fermenter containing 20 liters of YMG medium with aeration (2 liters air/minute) and agitation (120 rpm) at 22°C. After 3 days of fermentation the culture fluid was separated from the mycelium by filtration. The mycelium was extracted with methanol : acetone (1 : 1) and separated by chromatography on silica gel (Merck 60) with cyclohexane : EtOAc (1 : 1) as eluent. Preparative HPLC (Merck Lichrosorb DIOL, column 2.5 × 25 cm) with cyclohexan : *t*-butylmethylether (60 : 40) as eluent yielded 18 mg sesquicillin.

Spectroscopy

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) were recorded at room temperature in $CDCl_3$ with a Bruker ARX 500 spectrometer with an inverse 5 mm probe equipped with a shielded gradient coil. COSY, HMQC and HMBC experiments were performed with gradient enhancements using sine shaped gradient pulses, and for the 2D heteronuclear correlation spectroscopy the refocusing delays were optimised for $^1J_{CH} = 145$ Hz and $^2J_{CH} = 10$ Hz. The raw data were transformed and the spectra were evaluated with the standard Bruker UXNMR software (rev. 941001). The chemical shifts are given in ppm (with the solvent peaks at 7.26 and 77.0 ppm serving as reference) and the coupling constants J in Hz. EI mass spectra were recorded by a JEOL SX102 spectrometer at 70 eV, while the UV and the IR spectra were recorded with a Perkin Elmer λ 16 and a Bruker IFS 48 spectrometer. The melting point (uncorrected) were determined with a Reichert microscope, and the optical rotation measured with a Perkin-Elmer 141 polarimeter at 22°C.

Sesquicillin (**1a**) was obtained as white crystals, m.p. 165~167°C. $[\alpha]_D$ +10° (*c* 0.8 in $CHCl_3$). UV (MeOH) λ (nm) (ε): 290 (10,900). IR (KBr): 3425, 2930, 1735, 1670, 1560, 1450, 1385, 1240, 1115, 1075 and 1030 cm^{-1}. See Table 1 for ^{1}H and ^{13}C NMR data. EI-MS, *m/z*: 470.3055 (M^+, 18%, $C_{29}H_{42}O_5$ requires 470.3032), 395 (8%), 329 (17%), 299 (19%), 205 (29%), 153 (100%), 93 (53%), 69 (66%), 55 (80%), 45 (55%).

Acetylsesquicillin (**1b**) was obtained in quantitative yield after acetylation of sesquicillin (**1a**) with acetic anhydride in pyridine, as a colourless oil. $[\alpha]_D < \pm 1°$ (*c* 0.1 in $CHCl_3$). UV and IR spectra were not recorded. ^{1}H NMR ($CDCl_3$, 500 MHz), δ, mult. J (Hz): 5.04, t,

Table 1. ^1H (500 MHz) (δ; multiplicity; J) and ^{13}C (125 MHz) NMR data (δ; multiplicity) for sesquicillin (**1a**) in $CDCl_3$, with the $CHCl_3$/$CDCl_3$ signals (7.26 and 77.0 ppm) as references.

H/C	^1H data	^{13}C data
1a	1.96; m	33.8; t
1b	1.29; m	—
2a	1.75; m	24.0; t
2b	1.71; m	—
3	4.81; dd; 10.1, 5.9	76.2; d
4	—	40.0; s
5	1.68; dd; 2.5, 12	39.3; d
6a	1.56; m	22.6; t
6b	1.39; dddd; 12, 14, 12, 5	—
7a	2.41; ddd; 5, 12, 14	30.9; t
7b	2.22; m	—
8	—	149.0; s
9	1.94; m	56.5; d
10	—	37.6; s
11a	2.75; dd; 3.2, 14.0	22.2; t
11b	2.50; dd; 10.6, 14.0	—
12*E*	4.65; m	111.0; t
12*Z*	4.45; m	—
13	0.95; s	22.8; q
14	0.86; s	18.0; q
15a	1.27; m	37.8; t
15b	1.18; m	—
16a	1.97; m	21.8; t
16b	1.86; m	—
17	5.03; t; 7	124.4; d
18	—	131.4; s
19	1.66; s	25.7; q
20	1.58; s	17.5; q
1′	—	165.0; s
2′	—	103.0; s
3′	—	164.3; s
4′	—	106.0; s
5′	—	155.7; s
6′	1.90; s	9.9; q
7′	2.18; s	17.2; q
3-Ac	2.03; s	170.7; s/21.2; q

The coupling constants J are given in Hz.

$J_{16\sim17}=7$, 17-H; 4.78, dd, $J_{2a\sim3}=8.5$, $J_{2b\sim3}=7.2$, 3-H; 4.58, dd, $J_{7a\sim12E}=J_{12E\sim12Z}=2$; 12-H*E*; 4.36, m, 12-H*Z*; 2.53, m, 11-H_2; 2.35, m, 7-Ha; 2.28, s, 7′-H_3; 2.21, s, 6′-H_3; 2.15, m, 7-Hb; 2.08, m, 9-H; 2.03, s, 3-Ac-H_3; 1.97, m, 16-Ha; 1.83, m, 16-Hb; 1.82, m, 1-Ha; 1.79, s, 3′-Ac-H_3; 1.77, m, 2-Ha; 1.74, m, 2-Hb; 1.65, dd, $J_{5\sim6a}=3.0$, $J_{5\sim6b}=12.7$, 5-H; 1.67, s, 19-H_3; 1.58, s, 20-H_3; 1.56, m, 6-Ha; 1.37, dddd, $J_{5\sim6b}=13$, $J_{6a\sim6b}=13$, $J_{6b\sim7a}=13$, $J_{6b\sim7b}=5$, 6-Hb; 1.28; m, 1-Hb; 1.27, m, 15-Ha; 1.18, ddd, $J_{15a\sim15b}=14.5$, $J_{15b\sim16a}=12.2$, $J_{15b\sim16b}=4.6$, 15-Hb; 0.98, s, 13-H_3; 0.87, s, 14-H_3. ^{13}C NMR spectrum was not recorded. EI-MS, m/z: 512.3144 (M^+, 24%, $C_{31}H_{44}O_6$ requires 512.3138), 470 (4%), 452 (6%), 437 (8%), 371 (13%), 301 (19%), 257 (52%), 196 (22%), 175 (27%), 153 (100%).

Biological Assays

Cell culture, transfection and SEAP assay was done essentially as described previously[4]. SEAP expression was induced 16 hours posttransfection by addition of 100 nM of the synthetic glucocorticoid dexamethasone, with or without test compounds. Addition of dexamethason resulted in a 6~15 fold activation over the basal level of SEAP expression.

Results and Discussion

The molecular ion of sesquicillin is observed at m/z 470 in the EI-MS spectrum, and high resolution measurements suggested that the elemental composition of the compound is $C_{29}H_{42}O_5$. The unsaturation index of sesquicillin is consequently 9. Treatment with acetic anhydride in pyridine transforms sesquicillin to the diacetylated compound **1b**. The structure of **1a** could be determined by NMR experiments. The assignment of the ^1H and ^{13}C NMR signals was facilitated by COSY and HMQC experiments, while the carbon skeleton and the relative stereochemistry of sesquicillin was demonstrated by HMBC and NOESY experiments (pertinent HMBC and NOESY correlations are shown in Figure 1). The optical activity and the melting point of the sample isolated here are very similar to the values reported in the patents ($[\alpha]_D$ +11° (c 0.2 in $CHCl_3$)[3] and 168°C[2]). Several bioactive diterpenoids with a pyranone moiety that have been reported in the literature, examples are pycnophorin isolated from the fungus *Macrophoma kuwatsukai*[5] and the colletotrichins which are phytotoxins obtained from *Colletotrichum capsici*[6] and *C. nicotianae*[7].

COS-7 cells (ATCC CRL 1651) were transiently cotransfected with a reporter gene vector containing the SEAP under the control of 3 copies of the GRE, together with a plasmid expressing the human glucocorticoid receptor-α under the control of the CMV promoter. SEAP expression was induced 16 hours post transfection using 100 nM dexamethasone, with or without added test compounds. SEAP activity in the medium was measured 48 hours after transfection. Sesquicillin inhibits the glucocorticoid induced reporter gene expression with an IC_{50} of 0.1~0.5 μg/ml (see Figure 2). Compared to sesquicillin (**1a**), the inhibitory activity of acetylsesqui-

Fig. 1. Structure (relative configuration) and numbering of sesquicillin (**1a**) (left), and pertinent correlations observed in the HMBC (middle) and NOESY (right) spectra.

a: R=H; b: R=Ac.

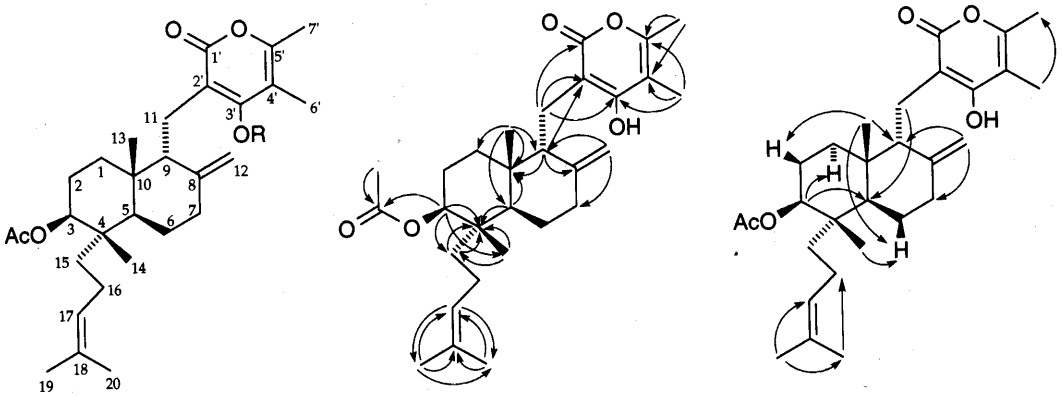

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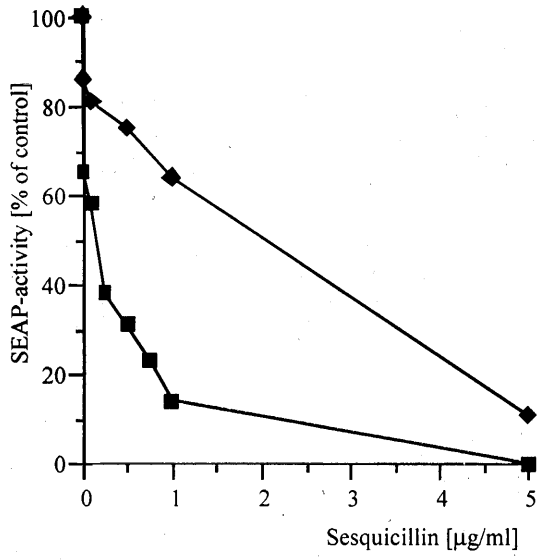
Fig. 2. Inhibition of glucocorticoid receptor and NF-κB mediated SEAP expression in COS-7 cells.

■ GRE DEX, ◆ NFκB TPA.



COS-7 cells were transfected with a reporter gene construct containing the SEAP gene under the control of the SV40 minimal promoter and 5× NF-κB consensus sequence or 3×GRE, respectively. For GRE mediated SEAP expression the cells have been cotransfected with a plasmid expressing the human glucocorticoid receptor α. Stimulation was performed by 50 ng/ml TPA (NF-κB) or 100 nM dexamethasone (GRE), with or without sesquicillin (**1a**). Control (100%): Stimulation without additions.

cillin (**1b**) on glucocorticiod dependent reporter gene expression is much less pronounced with IC_{50} values of $5 \sim 10\,\mu g/ml$, indicating the importance of the free C-3′ hydroxyl group. For the examination of the effects of sesquicillin on the SEAP expression under the control of the transcription factor NF-κB, COS-7 cells were transfected with the reporter plasmid pGE2-NF-κB[4]. Stimulation of the NF-κB mediated SEAP expression was performed by the addition of 50 ng/ml TPA. Inhibition of NF-κB mediated SEAP expression by sesquicillin is about 10 fold lower than the inhibition of glucocorticoid induced expression (IC_{50}: $2.5\,\mu g/ml$). Glucocorticoids are clinically important immunosupressive and anti-inflammatory agents, although the molecular mechanisms for these effects are poorly understood. McEwan *et al.*[8] suggest that the repression of inflammation is due to a direct interaction of the glucocorticoid receptor with other transcription factors, like NF-κB[9], what may explain the anti-inflammatory effect of sesquicillin. The precise mechanism of action of sesquicillin is currently under investigation.

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References

1) Barnes, P. J. & I. Adcock: Anti-inflammatory actions of steroids: molecular mechanisms. TIPS 14: 436~441, 1993

2) THIELE, B. & H. TSCHERTER (Sandoz Ltd.): Verfahren zur Herstellung eines neuen Metaboliten. DE 2,316,429, October 11th, 1973
3) KUWABARA, A.; S. FUJITA, S. KOBAYASHI & T. NISHIGORI (Nippon Kayaku KK, Japan): Sesquicillin isomer manufacture with *Acremonium*. JP 8,092,119, April 9th, 1996
4) ERKEL, G.; T. ANKE & O. STERNER: Inhibition of NF-κB activation by panepoxydone. Biochem. Biophys. Res. Commun. 226: 214～221, 1996
5) SASSA, T.; H. KATO & H. KAJIURA: Isolation and structure of pycnophorin, a novel diterpene α-pyrone with antibacterial activity, produced by phytopathogenic *Macrophoma kuwatsukai*. Tetrahedron Lett. 27: 2121～2124, 1986
6) GODDARD, R.; I. K. HATTON, J. A. K. HOWARD, J. MACMILLAN & C. J. GILMORE: X-Ray crystal and molecular structure of acetylcolletotrichin (colletotrichin), a metabolite of *Colletotrichum capsici*. J. Chem. Soc. Chem. Commun. 408～411, 1976
7) SUZUKI, A.; M. GOHBARA, Y. KOSUGE, S. TAMURA, Y. OHASHI & Y. SASADA: Structures of colletotrichin and colletotrichin B, phytotoxic metabolites from *Colletotrichum nicotianae*. Agric. Biol. Chem. 40: 2505～2506, 1976
8) MCEWAN, I. J.; A. P. H. WRIGHT & J.-A. GUSTAFSSON: Mechanism of gene expression by the glucocorticoid receptor: role of protein-protein interactions. BioEssays 19: 153～160, 1997
9) MANNING, A. M. & D. C. ANDERSON: Transcription factors: a new frontier for drug discovery. Annu. Rep. Med. Chem. 29: 235～244, 1996