VOL. 54 NO. 8, AUG. 2001 THE JOURNAL OF ANTIBIOTICS pp. 679 — 681

NOTES

New Bioactive Cyclopentenone Derivatives as Inhibitors of the IL-6 Dependent Signal Transduction

MARCUS WEIDLER[†], JAN RETHER[†], TIMM ANKE[†], GERHARD ERKEL[†,*] and OLOV STERNER[††]

[†] Institut für Biotechnologie und Wirkstoff-Forschung e.V. (IBWF) Erwin-Schrödinger-Str. 56, D-67663 Kaiserslautern, Germany
[††] Department of Organic Chemistry 2, Lund University, P.O. Box 124, S-221 00 Lund, Sweden

(Received for publication March 1, 2001)

The IL-6 dependent activation of the JAK/STAT pathway plays a central role in the induction of the acute-phase response in liver cells and stimulates the production of most acute phase proteins (APPs)[1,2]. Small molecule inhibitors interfering with the IL-6 dependent JAK/STAT signaling cascade leading to the expression of disease related genes would be expected to serve as novel therapeutic approaches[3]. In order to search for new inhibitors of the IL-6 dependent signaling, a cell based reporter assay in the hepatoma cell line HepG2 was used and submerged cultures of basidiomycetes, ascomycetes and imperfect fungi were examined for the production of compounds which interfere with the IL-6 induced JAK/STAT mediated expression of the reporter gene in the hepatoma cell line HepG2. A screening of 3100 strains of basidiomycetes, ascomycetes and fungi imperfecti resulted in the isolation of four novel cyclopenentone derivatives, 2-(1-chloro-1-propenyl)-4,5-dihydroxycyclopent-2-enone (CPDHC, **1**), 4,5-dihydroxy-2-propenylcyclopent-2-enone (DHPC, **2**), 5-hydroxy-2,3-dimethylcyclopent-2-enone (HDC, **3**) and 4,5-dihydroxy-2-methyl-1-methylenecyclopent-3-ene (MMCD, **4**) from fermentations of the ascomycete strain A23-98. In addition we investigated the influence of the isolated compounds on the expression of a human TNF-α transcriptional reporter in Jurkat cells. CPDHC (**1**) and DHPC (**2**) inhibited the TPA/ionomycin stimulated expression of the hTNF-α promoter mediated luciferase expression in Jurkat cells with IC_{50}-values of 5~10 μM (1~2 μg/ml) and 50~65 μM (8~10 μg/ml)[4]. No inhibitory activities on the hTNF-α driven reporter gene expression could be observed for the compounds HDC (**3**) and MMCD (**4**). In this paper we report the structural elucidation and some biological activities of the compounds.

Experimental

General Experimantal Section

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) were recorded at room temperature in $CDCl_3$ or in $CDCl_3$-CD_3OD (9 : 1) 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. In addition to the standard Bruker XWINNMR software, the Gifa software package was used for the evaluation of the NMR spectra[5]. The chemical shifts are given in ppm (with the solvent peaks for $CHCl_3$ and $CDCl_3$ at 7.26 and 77.0 ppm as reference) and the coupling constants J in Hz. EI and APCI mass spectra were acquired on a Finnigan Polaris and a Hewlett-Packard MSD 1100 spectrometer, while the UV and IR spectra were recorded with a Perkin Elmer Lambda 20 and a Perkin Elmer Spektrum One B spectrometer. The melting points (uncorrected) were determined with a Reichert microscope, and the optical rotation measured with a Perkin Elmer 141 polarimeter at 22°C.

2-(1-Chloro-1-propenyl)-4,5-dihydroxycyclopent-2-enone (CPDHC, 1)

2-(1-Chloro-propenyl)-4,5-dihydroxycyclopent-2-enone was obtained as pale yellow crystals, m.p. 84~86°C. $[\alpha]_D$ −5° (c 0.1 in MeOH). UV (MeOH) λ (nm) (ε): 226 (7,950). IR (KBr): 3392, 2918, 1718, 1639, 1324, 1276, 1237, 1149, 1059 and 1030 cm^{-1}. EI-MS, m/z: 190 (^{37}Cl−M$^+$, 14%), 188.0255 (^{35}Cl−M$^+$, 44%, $C_8H_9O_3{}^{35}Cl$ requires 188.0240), 173 (17%), 159 (19%), 155 (31%), 153 (100%), 135 (61%), 125 (21%), 107 (32%). ^{1}H NMR ($CDCl_3$: CD_3OD 9 : 1, 500 MHz), δ, mult., J (Hz): 7.28, d, $J_{3\sim4}$=1.8, 3-H; 7.02, q, $J_{2'\sim3'}$=6.9, 2′-H; 4.55, m, 4-H; 4.12, d, $J_{5\sim4}$=3.0, 5-H; 1.84, d, $J_{3'\sim2'}$=6.9, 3′-H_3. ^{13}C NMR

* Corresponding author: erkel@ibwf.uni-kl.de

($CDCl_3$: CD_3OD 9 : 1, 125 MHz), δ: 199.9 C-1; 153.2 C-3; 137.9 C-2; 130.0 C-2′; 124.2 C-1′; 82.9 C-5; 73.6 C-4; 14.6 C-3′.

4,5-Dihydroxy-2-propenylcyclopent-2-enone (DHPC, **2**)

4,5-Dihydroxy-2-propenylcyclopent-2-enone was obtained as a 1 : 1 mixture of the 4,5-*cis*-dihydroxy and 4,5-*trans*-dihydroxy isomers, as pale yellow crystals, m.p. 112~117°C. UV (MeOH) λ (nm) (ε): 207 (15,600). IR (KBr): 3402, 3028, 2917, 1721, 1637, 1417, 1295, 1223, 1140 and 1055 cm^{-1}. EI-MS, *m/z*: 154.0631 (M^+, 10%, $C_8H_{10}O_3$ requires 154.0630), 139 (29%), 136 (46%), 126 (12%), 125 (13%), 121 (44%), 111 (18%), 108 (23%), 107 (30%), 79 (100%). ^1H NMR (*cis* isomer, $CDCl_3$, 500 MHz), δ, mult., *J* (Hz): 7.45, d, $J_{3\sim4}$=3.3, 3-H; 6.05, m, 1′-H and 2′-H; 4.91, dd, $J_{4\sim5}$=5.6, $J_{4\sim3}$=3.3, 4-H; 4.20, d, $J_{5\sim4}$=5.6, 5-H; 1.87, d, $J_{3'\sim2'}$=6, 3′-H_3. ^{13}C NMR (*cis* isomer, $CDCl_3$, 125 MHz), δ: 206.1 C-1; 153.4 C-3; 140.5 C-2; 134.6 C-2′; 117.5 C-1′; 71.2 C-5; 67.8 C-4; 15.8 C-3′. ^1H NMR (*trans* isomer, $CDCl_3$, 500 MHz), δ, mult., *J* (Hz): 7.25, d, $J_{3\sim4}$= 1.9, 3-H; 6.05, m, 1′-H and 2′-H; 4.81, m, 4-H; 4.26, d, $J_{5\sim4}$=2.7, 5-H; 1.87, d, $J_{3'\sim2'}$=6, 3′-H_3. ^{13}C NMR (*trans* isomer, $CDCl_3$, 125 MHz), δ: 203.5 C-1; 153.1 C-3; 139.1 C-2; 134.2 C-2′; 117.2 C-1′; 81.5 C-5; 75.5 C-4; 15.8 C-3′.

5-Hydroxy-2,3-dimethylcyclopent-2-enone (HDC, **3**)

5-Hydroxy-2,3-dimethylcyclopent-2-enone was obtained as pale yellow crystals, m.p. 40~45°C. $[\alpha]_D$ −29° (*c* 0.2 in MeOH). UV (MeOH) λ (nm) (ε): 239 (7,950). IR (KBr): 3427, 2925, 1704, 1639, 1517, 1437, 1392, 1336, 1098 and 1048 cm^{-1}. EI-MS, *m/z*: 126.0690 (M^+, 100 %, $C_7H_{10}O_2$ requires 126.0681), 111 (41%), 109 (17%), 107 (45%), 83 (21%). ^1H NMR ($CDCl_3$, 500 MHz), δ, mult., *J* (Hz): 4.17, dd, $J_{5\sim4a}$=6.7, $J_{5\sim4b}$=2.9, 5-H; 2.83, dd, $J_{4a\sim5}$=6.7, $J_{4a\sim4b}$=17.8, 4-Ha; 2.40, dd, $J_{4b\sim5}$=3, $J_{4b\sim4a}$=17.8, 4-Hb; 2.02, s, 3-CH_3; 1.67, s, 2-CH_3. ^{13}C NMR ($CDCl_3$, 125 MHz), δ: 209.6 C-1; 168.6 C-3; 133.7 C-2; 71.0 C-5; 40.2 C-4; 17.1 3-CH_3; 7.7 2-CH_3.

4,5-Dihydroxy-2-methyl-1-methylenecyclopent-3-ene (MMCD, **4**)

4,5-Dihydroxy-2-methyl-1-methylenecyclopent-3-ene was obtained as pale yellow crystals, m.p. 90~92°C. $[\alpha]_D$ +265° (*c* 0.1 in MeOH). UV (MeOH) λ (nm) (ε): 234 (14,250). IR (KBr): 3235, 2941, 1645, 1622, 1437, 1325, 1227, 1147, 1076 and 1053 cm^{-1}. EI-MS, *m/z*: 126.0677 (M^+, 35%, $C_7H_{10}O_2$ requires 126.0681), 125 (28%), 111 (100%), 109 (38%), 79 (32%). ^1H NMR ($CDCl_3$, 500 MHz), δ, mult., *J* (Hz): 5.72, bs, 3-H; 5.06, bs, 6-Ha; 4.99, bs, 6-Hb; 4.59, d, $J_{1\sim2}$=2, 4-H; 4.46, d, $J_{2\sim1}$=2, 5-H; 1.80, s, 2-CH_3. ^{13}C NMR ($CDCl_3$, 125 MHz), δ: 153.6 C-1; 141.4 C-2; 132.2 C-3; 104.2 1-CH_2; 82.0 C-5; 81.8 C-4; 12.8 2-CH_3.

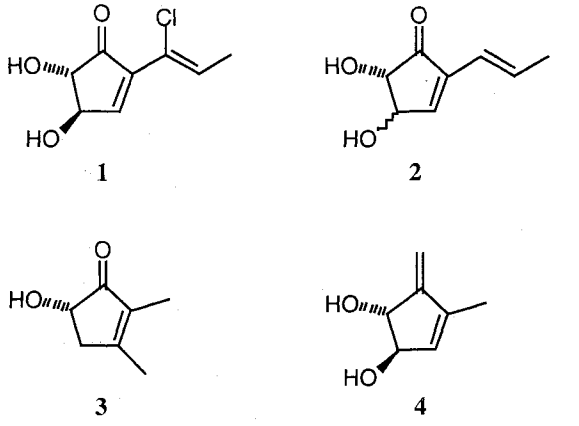
Fermentation, Isolation and Biological Assays

The ascomycete strain A23-98 was isolated from a wood sample collected in La Reunion. The species could not be identified. The strain was kindly provided by H. ANKE and is deposited in the culture collection of the LB Biotechnologie, Universität Kaiserslautern. The maintenance of the producing strain, the fermentation and isolation of the compounds **1**~**4** was performed as recently described[4]. The assays for antimicrobial activity and cytotoxicity were carried out as described recently[6]. Transcritional activity of a class II IL-6 responsive element (IL-6RE II) driven SEAP or a hTNF-α promotor driven luciferase reporter plasmid was measured in transiently transfected HepG2 or Jurkat cells as previously described[4].

Results and Discussion

CPDHC (**1**) was isolated from a crude extract of the culture fluid of the ascomycete A23-98 by bioactivity-guided fractionation[4]. High resolution MS experiments suggested that the elemental compositions of compounds **1**, **3** and **4** are $C_8H_9O_3Cl$, $C_7H_{10}O_2$ and $C_7H_{10}O_2$, respectively, and this was confirmed by the isotope pattern in the mass spectrum of **1** and by the 1D NMR data. Compound **2** is an epimeric mixture (1 : 1), the mass spectra of the mixture suggested that both components have the elemental composition $C_8H_{10}O_3$ and the NMR spectra showed two similar set of signals corresponding to $C_8H_{10}O_3$. In **1** and in one of the isomers of **2** the coupling constant between the olefinic ring proton (3-H) and 4-H is small, less than 2 Hz, and so is the coupling constant between 4-H and 5-H (approximately 3 Hz). In the other isomer of **2** these coupling constants are bigger, 3.3 and 5.6 Hz, suggesting that the relative stereochemistry of the dihydroxy-cyclopentenone moiety is different. A strong NOESY correlation between 4-H and 5-H in the latter isomer of **2** suggests that the two hydroxyl groups are *cis* in this isomer, and this is supported by the corresponding coupling constant reported for 4,5-*trans*-dihydroxy-3-methyl-2-cyclopentenone isolated from an alga[7]. The absolute stereochemistry of this product was determined by total synthesis, the same absolute configuration is shown for the metabolites isolated here (Fig. 1) although this was not determined. The configuration of the pentenyl double bond in **1** was suggested to be *Z* by the lack of NOESY

Fig. 1. Structures of 2-(1-chloro-1-propenyl)-4,5-dihydroxycyclopent-2-enone (CPDHC, **1**), 4,5-dihydroxy-2-propenylcyclopent-2-enone (DHPC, **2**), 5-hydroxy-2,3-dimethylcyclopent-2-enone (HDC, **3**) and 4,5-dihydroxy-2-methyl-1-methylenecyclopent-3-ene (MMCD, **4**).



correlations between 3-H and both 1′-H and 3′-H_3. In **2**, the signals for 1′-H and 2′-H in both isomers have the same chemical shift in the ^{1}H NMR spectrum, and it was not possible to extract the coupling constants for these protons. However, the lack of NOESY correlations between 3′-H_3 in both isomers and protons in the cyclopentenone ring suggests that the double bond of the propenyl group has an *E* configuration. While **1**, **2** and **3** are cyclopentenone derivatives **4** is a methylencyclopenten derivative, but it is nevertheless probable that all four have the same biogenetic origin. The structurally related compounds 3-chloro-4-hydroxy-2-propenylcyclopent-2-enecarboxylic acid methyl ester and 2,3-dihydroxy-4-propenylcyclopentanone have been shown to be formed *via* the pentaketide pathway from dihydroisocoumarin[8], and it is possible this also is the case with the compounds reported here.

HepG2 cells were transiently transfected with a reporter gene vector containing the SEAP under control of multiple IL-6RE II sites, whereas Jurkat cells were transfected with a human TNF-α promoter driven luciferase plasmid. CPDHC (**1**) inhibited the IL-6 induced SEAP expression with IC_{50} values of 4.0~5.3 μM (0.75~1 μg/ml). The compounds DHPC (**2**), HDC (**3**) and MMCD (**4**) which are structurally closely related to CPDHC (**1**) showed no inhibitory effects on the IL-6 induced SEAP expression in HepG2 cells. DHPC (**2**) exhibited no and CPDHC (**1**) only weak antibacterial, antifungal or cytotoxic effects at comparatively high concentrations (>64 μg/ml). HDC (**3**) and MMCD (**4**) showed no biological activities up to 128 μg/ml. Further biological activities of **1** and **2** as well as the mechanism of action of CPDHC (**1**) in inhibiting the IL-6 dependent signal transduction have recently been published[4].

Acknowledgements

This work was supported by a grant from the Stiftung Rheinland-Pfalz für Innovation (8312-38 62 61/322a) and the Bundesministerium für Bildung und Forschung. We thank H. ANKE for providing the ascomycete strain A23-98 and N. HEIL for skillful technical assistance.

References

1) HEINRICH, P. C.; I. BEHRMANN, G. MÜLLER-NEWEN, F. SCHAPER & L. GRAEVE: Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. Biochem. J. 334: 297~314, 1998
2) JENSEN, L. E. & A. S. WHITEHEAD: Regulation of serum amyloid A protein expression during the acute-phase. Biochem. J. 334: 489~503, 1998
3) LAMB, P.; P. TAPLEY & J. ROSEN: Biochemical approaches to discovering modulators of the JAK-STAT pathway. Drug Discov. Today 3: 122~130, 1998
4) WEIDLER, M.; J. RETHER, T. ANKE & G. ERKEL: Inhibition of interleukin-6 signaling and Stat3 activation by a new class of bioactive cyclopentenone derivatives. Biochem. Biophys. Res. Commun. 276: 447~453, 2000
5) PONS, J. L.; T. E. MALLIAVIN & M. A. DELSUC: Gifa V4: a complete package for NMR data-set processing. J. Biomol. NMR 8: 445~452, 1996
6) WEBER, W.; T. ANKE, B. STEFFAN & W. STEGLICH: Antibiotics from basidiomycetes. XXXII. Strobilurin E: a new cytostatic and antifungal (E)-beta-methoxyacrylate antibiotic from *Crepidotus fulvotomentosus* Peck. J. Antibiotics 43: 207~212, 1990
7) OHIRA, S.; H. NOZAKI, M. ANDO, F. SHIRANE, H. KONDO & M. NAKAYAMA: Absolute structure of (−)-4,5-*trans*-dihydroxy-3-methyl-2-cyclopentenone isolated from the marine alga, *Grateloupia filicina* (Wulfen) J. Agardh by the synthesis of both enantiomers. Agric. Biol. Chem. 55: 2437~2438, 1991
8) HERBERT, R. B.: The biosynthesis of secondary metabolites. London: Chapman and Hall, 1989