Nidulal, a Novel Inducer of Differentiation of Human Promyelocytic Leukemia Cells from *Nidula candida*

GERHARD ERKEL*, UTA BECKER and TIMM ANKE

Lehrbereich Biotechnologie der Universität Kaiserslautern,
Paul-Ehrlich-Str. 23, D-67663 Kaiserslautern, Germany

OLOV STERNER

Division of Organic Chemistry 2, Lund University,
S-221 00 Lund, Sweden

(Received for publication May 27, 1996)

Nidulal (**1**), a novel inducer of differentiation of human HL-60 promyelocytic leukemia cells, was isolated from fermentations of the basidiomycete *Nidula candida* together with low amounts of niduloic acid (**2**). Both compounds are bisabolane sesquiterpenes. Their structures were elucidated by spectroscopic methods. In reporter gene assays nidulal (**1**) preferentially activated the transcription factor complex AP-1 - mediated expression of secreted alkaline phosphatase in COS-7 cells. In addition nidulal (**1**) and niduloic acid (**2**) exhibited weak cytotoxic and antibiotic activities.

Tumor cells differ from their normal counterparts by their ability to evade the regulatory mechanisms of their environment and to propagate in an uncontrolled fashion. Among the phenotypic abnormalities in acute leukemia is a lack of granulocytes, macrophages and platelets caused by the inability of the neoplastic leucocytes to undergo terminal differentiation and eventually apoptosis. The human HL-60 leukemia cell line is an excellent model for a study functional and morphological differentiation *in vitro*, because the cells can be induced to differentiate into granulocytes or monocytes/macrophages[1]. Differentiation may be followed by apoptosis, a process of active DNA fragmentation[2]. The induction of differentiation and apoptosis are regulated by a network of signal transduction pathways and transcription factors which are possible targets for a rational antitumor therapy[3]. In the course of a screening for fungal metabolites inducing the differentiation of HL-60 cells, the new compound nidulal (**1**) and low amounts of another new bisabolane sesquiterpene niduloic acid (**2**) were isolated from cultures of the basidiomycete *Nidula candida*. In this paper the fermentation of *Nidula candida*, the isolation, physicochemical properties and structure determination of nidulal (**1**) and niduloic acid (**2**) (Fig. 3), as well as biological properties of nidulal (**1**) are described.

Materials and Methods

General

Spectral data were recorded on the following instruments: UV, Perkin-Elmer λ16; IR, Bruker IFS48; EI-MS and HREI-MS, Jeol JMS-SX102 spectrometer (direct inlet, 70 eV); NMR, Bruker ARX 500. The ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded at room temperature 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 optical rotation was measured with a Perkin Elmer 141 polarimeter with a cell path of 10 cm. Melting points, which are uncorrected, were determined using a Reichert microscope, and TLC experiments were performed on Merck silica gel 60 F254 precoated plates.

Producing Organism

Fruiting bodies of *Nidula candida*, strain 87220 were collected in Port Albani, Canada. They show all characteristics of the genus and species[4]. The strain is deposited in the culture collection of the LB Biotechnologie, Universität Kaiserslautern.

Fermentation

For maintenance on slant agars and submerged cultivation, *Nidula candida* 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. A well grown seed culture of *Nidula candida* (200 ml) in YMG was used to inoculate 20 liters of YMG medium in a Biolafitte C6 fermenter. The fermenter was incubated

at 22°C with an aeration rate of 3 liters air/minute and agitation (120 rpm). The production of active compounds was followed by measuring the induction of differentiation of HL-60 cells by various concentrations of a crude extract.

Isolation

After 4 days the culture broth (18 liters) was extracted with EtOAc (10 liters). Evaporation of the organic phase yielded a crude extract (2.6 g) which was applied to a column (2.5 × 20 cm) containing silica gel (Merck 60; 5 × 15 cm). After elution with cyclohexane - EtOAc (75 : 25) an enriched product (600 mg) was obtained. Bioassay-guided (differentiation assay with HL-60 cells) fractionation of this product by preparative HPLC (LiChrosorb CN, column 2.5 × 25 cm) with cyclohexane : *tert*-butyl methyl ether 1 : 3 as eluant) yielded pure nidulal (**1**) (51 mg), and in addition niduloic acid (**2**) (1.2 mg).

Biological Assays

The antimicrobial spectra, cytotoxicity and macromolecular syntheses in whole HL-60 cells (ATCC CCL 240) were measured as described previously[5]. The effect on cell growth of monolayer cell lines was measured with Giemsa stain as described by ERKEL[6]. Cell viability of suspension cell lines was measured by the XTT test (Boehringer Mannheim) as described in the product information. HeLa S3 cells (ATCC CCL 2.2), BHK-21 cells (ATCC CCL 10), COS-7 cells (ATCC CRL 1651) and RBL-1 cells (ATCC CRL 1378) were grown in D-MEM medium, L-1210 cells (ATCC CCL 219) were grown in HAM's F 12 medium and HL-60 and U-937 (ATCC CRL 1593) cells in RPMI-1640 supplemented with 10% fetal calf serum and 65 μg/ml benzylpenicillin and 100 μg/ml streptomycin sulfate in a humidified atmosphere containing 5% of CO_2 at 37°C.

Mutagenicity was tested as described by AMES *et al.*[7]. Mutants of *Salmonella typhimurium*, strain TA 98 and strain TA 100 were used for the spot test with and without rat liver microsomes.

The induction of morphological and physiological differentiation of HL-60 cells was assayed as described previously[8]. The cells were grown for 4 days with or without the compounds to be tested. Differentiated cells reduced the water-soluble nitro-blue tetrazolium chloride (NBT) to blue-black cell-associated nitro-blue diformazan deposits. For quantification the percentage of stained cells was determined. The induction of morphological and physiological differentiation of U-937 cells was assayed as described for HL-60 cells with the following modifications: The concentration of phorbolester (TPA) in the NBT dye solution was increased to 10 mg/ml and the incubation time with the dye solution was increased to 60 ~ 120 minutes. Apoptosis was measured as described by NAGY *et al.*[9] with slight modifications: HL-60 cells (5×10^5/ml in a 24-well tissue culture plate) were incubated with the substances for 16 or 96 hours and collected by centrifugation at 200 × *g* for 10 minutes at 4°C. After washing with phosphate buffered saline (PBS) the cells were lysed by the addition of 200 μl of 20 mM Tris-HCl (pH 7.4) - 0.4 mM EDTA - 0.4% Triton X-100, transferred to a microcentrifuge tube, and centrifugated at 4,000 × *g* for 10 minutes at 4°C. The supernatants were collected, adjusted to 0.5 M NaCl (final concentration), and the DNA was precipitated by addition of 250 μl isopropanol. The DNA was collected by centrifugation at 15,000 × *g* for 30 minutes, washed once with 70% ethanol and the samples were separated by electrophoresis on a 1.5% agarose gel.

Reporter gene assays: The reporter plasmids pGE2-NF1, pGE2-AP1, pGE2-CREB and pGE2-GRE contain the reporter gene secreted alkaline phosphatase (SEAP) under the control of an enhancerless SV40 promotor and five copies of the NF-κB (pGE2-NF1), three copies of the AP-1 (pGE2-AP1), six copies of the c-AMP responsive element binding site (pGE2-CREB) and three copies of the glucocorticoid responsive element (pGE2-GRE) binding sites, respectively[10]. In addition, activation of the glucocorticoid inducible reporter gene expression has been determined by cotransfection of the human glucocorticoid receptor-α (pRShGR, ATCC 67200). Transfections of COS-7 have been performed by electroporating 3×10^6 cells suspended in 1 ml phosphate buffered saline (PBS) containing 30 μg of the reporter constructs at 500 V/cm and $\tau = 20 \sim 23$ ms using a gene pulser apparatus (BioRad). After electroporation the cells were seeded at 1×10^5 cells/ml Opti-MEM (GIBCO, BRL) containing 10% FCS in a 24 well tissue culture plate and allowed to recover for 16 hours. For induction of SEAP expression, cells were treated with various concentrations of test compounds in Opti-MEM containing 0.5% FCS and the activity of the SEAP in the culture medium was determined 60 hours after transfection using the Phospha-Light chemiluminiscent reporter gene assay (TROPIX, MA) according to the manufacturer's instructions with a liquid scintillation counter.

Electrophoretic mobility shift assays (EMSA): HL-60 cells were starved for 16 hours in RPMI-medium with 0.5% FCS, treated for 16 hours with nidulal or induced with 2 ng/ml TNF-α or various concentrations of TPA. Total cell extracts were prepared using a high-salt detergent buffer and tested for binding to a high consensus AP-1 binding sequence (Promega, Madision, U.S.A.) as recently described[11].

Nidulal (**1**)

Compound **1** was obtained as a colourless oil. $[\alpha]_D^{22}$ 0° (*c* 0.7 in chloroform). Rf-values: 0.51 (SiO_2/toluene : acetone : acetic acid, 70 : 30 : 1) and 0.31 (SiO_2/cyclohexane : ethyl acetate, 1 : 1). UV (MeOH) λ_{max} (ε): 273 (6000). IR (Kbr): 2934, 1793, 1722, 1611, 1452, 1256, 1126, 983, 851, 754 cm^{-1}. ^1H NMR (500 MHz in $CDCl_3$), δ, multiplicity, *J* (Hz): 9.63, d, $J_{3\sim15} = 1.1$, 15-H; 8.00, d, $J_{6\sim14} = 0.7$, 14-H; 6.63, q, $J_{10\sim13} = 1.6$, 10-H; 2.29,

m, 6-H; 2.22, m, 3-H; 2.08, m, 2-H_{eq} and 4-H_{eq}; 2.05, m, 1-H_{eq} and 5-H_{eq}; 2.02, d, $J_{10\sim13}=1.6$, 13-H_3; 1.37, m, 2-H_{ax} and 4-H_{ax}; 1.35, m, 1-H_{ax} and 5-H_{ax}. ^{13}C NMR (125 MHz in $CDCl_3$), d: 203.8 C-15; 194.9 C-8; 172.2 C-14; 170.2 C-12; 139.0 C-10; 135.9 C-11; 123.5 C-7; 102.6 C-9; 49.5 C-3; 31.6 C-6; 30.0 and 29.9 C-1 and C-5; 25.6; C-2 and C-4; 10.9 C-13. MS (EI, 70 eV), *m/z*: 276.0981 (M^+, 22%, $C_{15}H_{16}O_5$ requires 276.0998), 258 (15%), 252 (14%), 195 (12%), 168 (80%), 96 (100%), 73 (82%), 68 (86%).

Niduloic acid (**2**)

Compound **2** was obtained as yellow oil. $[\alpha]_D^{22}$ $-53°$ (*c* 0.3 in chloroform : methanol 9 : 1). Rf-values: 0.31 (SiO_2/toluene : acetone : acetic acid 70 : 30 : 1). UV (EtOH) λ_{max} (ε): 225 (7200), 278 (2600). IR (KBr): 2930, 1792, 1684, 1653, 1609, 1456, 1419, 1258, 946, 732 cm^{-1}. ^1H NMR (500 MHz in $CDCl_3$), δ, multiplicity, *J* (Hz): 7.07, s, 14-H; 6.93, m, 2-H; 6.77, s, 8-H; 6.34, m, 10-H; 2.61, m, 6-H; 2.43, dm, $J_{1a\sim1b}=18$, 1-Ha; 2.33, dm, $J_{1a\sim1b}=18$, 4-Ha; 2.19, m, 4-Hb; 2.12, m, 1-Hb; 1.97, d, $J_{10\sim13}=1.2$, 13-H_3; 1.92, m, 5-Ha; 1.51, m, 5-Hb. ^{13}C NMR (125 MHz in $CDCl_3$), d: 170.6 C-12; 169.5 C-15; 150.8 C-9; 139.0 C-2; 138.1 C-14; 131.6 C-7; 130.0 C-3; 125.3 C-11; 123.8 C-10; 112.4 C-8; 32.4 C-1; 29.5 C-6; 28.6 C-5; 23.7 C-4; 21.5 C-13. MS (EI, 70 eV), *m/z*: 276.1006 (M^+, 100%, $C_{15}H_{16}O_5$ requires 276.0998), 258 (9%), 230 (16%), 213 (14%), 178 (94%), 96 (89%), 84 (82%).

Results

Production of Nidulal (**1**) and Niduloic Acid (**2**)

The production of nidulal (**1**) and niduloic acid (**2**) started 2~3 days after inoculation of fermentations of *Nidula candida* (Fig. 1). The activity of the crude extract in the differentiation assay peaked after approximately 4 days. The extraction of the culture broth and isolation of nidulal (**1**) is described in the experimental section. In addition to **1**, smaller amounts of niduloic acid (**2**) were obtained from the same chromatography fraction.

Structural Elucidation of Nidulal (**1**)

The structures of nidulal (**1**) and niduloic acid (**2**) are shown in Fig. 2. Mass spectroscopic analyses of nidulal (**1**) suggested that its elemental composition is $C_{15}H_{16}O_5$ which is in agreement with the 1D NMR data (given in Materials and Methods). The structure should therefore have 8 unsaturations. The elucidation of the structure is based on the correlations observed in 2D NMR spectroscopy (COSY, HMQC, HMBC and NOESY), of which the pertinent HMBC correlations are shown in Fig. 3. The signals for the cyclohexane protons were not well resolved in the ^1H NMR spectrum, possibly due to the restriction in the free rotation around C-6/C-7 by the C-8 keto function. This makes C-1 slightly different from C-5, their ^{13}C resonances differ by 0.1 ppm, and the cyclohexane ring is not completely symmetrical. The aldehydic proton gives a characteristic small ^1H-^1H coupling to 3-H, which in turn couples with 2-H_2 and/or 4-H_2. The ^1H spin system continues from 2-H_2/4-H_2 *via* 1-H_2/5-H_2 to 6-H. There is no observable difference between the chemical shifts for 2-H_2 and 4-H_2, or for 1-H_2 and 5-H_2, and the shifts for C-2 and C-4 are identical making the HMBC correlation from 15-H to C-2 and/or C-4 hazardous. However, HMBC correlations can be observed between 2-H_2/4-H_2 and C-2/C-4, and between 1-H_2/5-H_2 and C-1/C-5, supporting a 1,4-disubstituted cyclohexane structure. A correlation from 6-H to C-7 can be observed in the HMBC spectrum, and in addition also weak correlations from 6-H to C-8 and C-14. A long-range COSY correlation between 6-H and 14-H (0.7 Hz) as well as HMBC correlations between 1-H_2/5-H_2 and C-7, and between 14-H and C-7 as well

Fig. 1. Fermentation of *Nidula candida* in 20 liters YMG medium.

□ pH; ∗ mycelial dry weight (g/liter); ○ maltose (g/liter); △ glucose (g/liter); ◇ NBT-reduction: differentiated cells (%) caused by 50 μg/ml crude extract.

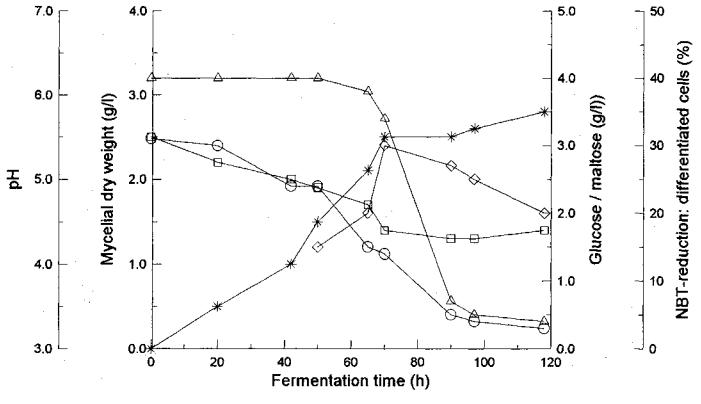


Fig. 2. Structures of nidulal (**1**) and niduloic acid (**2**).

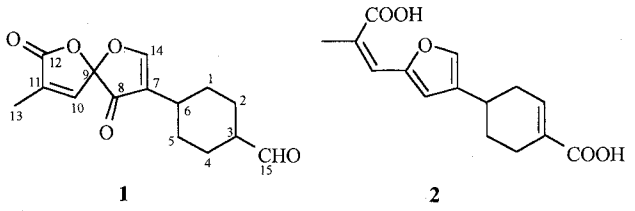
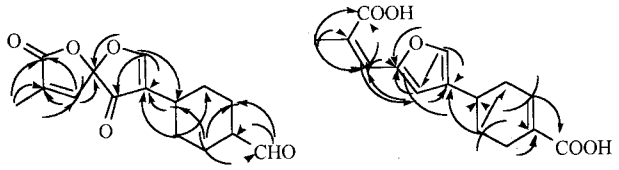


Fig. 3. Pertinent HMBC correlations observed with nidulal (**1**) and niduloic acid (**2**).



as C-6 support the suggested attachment of the C-7/C-14 double bond to C-6. The chemical shifts for 14-H (8.00 ppm) and C-14 (172.2 ppm) as well as the large $^1J_{CH}$ (196 Hz) observed in the coupled ^{13}C NMR spectrum support that C-14 is both oxygenated and the b-carbon of an a,b-unsaturated ketone. Besides the HMBC correlations from 14-H to C-6, C-7 and C-8, a correlation is also observed to C-9. The chemical shift of C-9 (102.6 ppm) suggests that it is an acetal carbon, and a HMBC correlation to C-9 is also observed from 10-H. An allylic ^1H-^1H coupling between 10-H and 13-H_3, and HMBC correlations between 13-H_3 and C-10, C-11 and C-12 as well as between 10-H and C-11 and C-12, shows that the final fragment of nidulal (**1**) must be a lactol ring with C-9 as the acetal carbon. It has not been possible to assign the relative stereochemistry of nidulal (**1**). The two substituents on the cyclohexane ring are trans and equatorial, as suggested by the NOESY correlations between 3-H and 1-H_{ax}/5-H_{ax} as well as 6-H and 2-H_{ax}/4-H_{ax}, but from NMR data it is not possible to determine the configuration of C-9 compared to C-6. Since nidulal shows no optical activity, it appears to be a racemic mixture.

Structural Elucidation of Niduloic Acid (2)

Niduloic acid (**2**) has the same molecular weight and elemental composition as nidulal (**1**), and the two compounds are obviously related. The 6-membered ring in the bisabolane skeleton is oxidised to a cyclohexene in compound **2**, as shown by the proton spin system from 2-H *via* 1-H_2, 6-H, and 5-H_2 to 4-H_2. HMBC correlations (Fig. 3) from 4-H_2 and 5-H_2 to C-3 show that the C-2/C-3 double bond is part of the 6-membered ring, and not exocyclic. C-15 is oxidised to a carboxylic acid in niduloic acid (**2**), and a correlation between 2-H and C-15 could be observed in the HMBC spectrum. The HMBC correlations from 6-H were all very weak, also to C-7, but long-range COSY correlation between 6-H and 8-H as well as 14-H (not resolved in the ^1H NMR spectrum) as well as NOESY correlations between both 8-H and 14-H and 1-H_2, 5-H_2 as well as 6-H show that the two olefinic protons are in the vicinity of C-6. 8-H also gives a HMBC correlation to C-6, even if this was not observed for 14-H. The chemical shifts for 8-H, 14-H, C-8 and C-14 suggest that they, together with C-7 and C-9 constitute a 2,4-disubstituted furan, and the HMBC correlations between 1-H_2/5-H_2 and C-7 show that this is attached to the cyclohexene ring at C-7. 14-H also gives a HMBC correlation over the furan oxygen to C-9, to which a a-methylacrylic acid moiety is attached. The correlations observed in this are similar to those discussed above for C-10/C-11/C-12/C-13 in nidulal (**1**). The observed NOESY correlations between 10-H and 8-H as well as 13-H_3 support the suggested structure, and the presence of two free carboxylic functions in niduloic acid (**2**) is indicated by the enhancement of its Rf-value in chromatographic systems containing small amounts of acid (see Materials and Methods).

Biological Properties

Nidulal (**1**) induced the differentiation of 15~25% of HL-60 cells at a concentration of 72 μM as measured by NBT reduction (Table 1). 1.5% DMSO, used as a standard, induced 70~80% of the HL-60 cells to differentiate. Treatment of HL-60 cells with TPA or TNF-α (2 ng/ml) resulted in an induction of differentiation of 60~70% and 25~35%, respectively. In the controls without additions 4~8% of HL-60 cells differentiated spontaneously. At the same concentration of (**1**) (72 μM) only 5~10% of U-937 cells differentiated (Table 2). Retinoic acid, used as a positive control, induced 30~40% of the cells to differentiate. Controls without induction contained 1~2% spontaneously differentiated U-937 cells.

Electrophoresis of total DNA from HL-60 cells treated with 72 μM nidulal revealed an induction of DNA fragmentation after 96 hours which was comparable to retinoic acid (5 μg/ml) as positive control (data not shown). In addition nidulal inhibited the proliferation of HL-60 and U-937 cells at concentrations starting from 72 μM up to 182 μM. Cellular DNA-, RNA-, and protein

Table 1. Differentiation of HL-60 cells after 96 hours of incubation.

Substance	Concentration	Differentiated cells (%)	Cell number (cells/ml)	Metabolic activity (%)
None	—	4~8	5×10^5	243
DMSO	1.5% (v/v)	70~80	1.3×10^5	100
TNF-α	2 ng/ml	25~35	1.7×10^5	156
TPA	1.6 nM	60~70	1.2×10^5	104
Retinoic acid	16 μM	70~80	1.3×10^5	107
Nidulal (**1**)	72 μM	15~25	1.3×10^5	98
Niduloic acid (**2**)	36 μM	15~25	1.2×10^5	115

Metabolic activity was measured by XTT reduction.

Table 2. Differentiation of U-937 cells after 96 hours of incubation.

Substance	Concentration (μM)	Differentiated cells (%)	Cell number (cells/ml)	Metabolic activity (%)
None	—	1~2	5×10^5	158
Retinoic acid	6.6	30~40	1.3×10^5	100
Nidulal (**1**)	72	5~10	1.3×10^5	138

Metabolic activity was measured by XTT reduction.

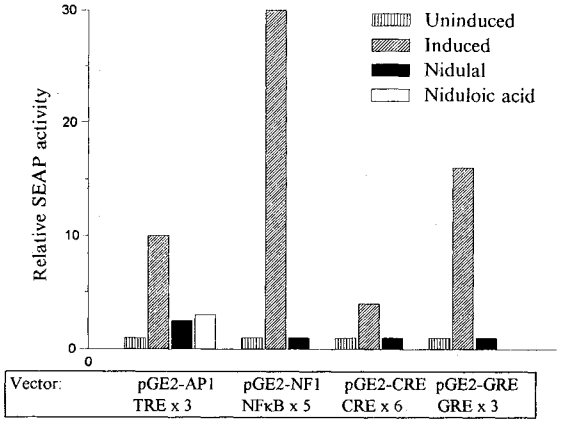
syntheses were examined in HL-60 cells by determining the incorporation of [^{14}C]-thymidine, [^{14}C]-uridine and [^{14}C]-leucine into acid-insoluble fractions. All three syntheses were inhibited about 50% at 182 μM of (**1**), which may account for the cytostatic properties (data not shown).

Transfection of COS-7 cells with an SEAP reporter gene plasmid containing the SV-40 minimal promotor and 3 × TPA response element (TRE) and induction with 50 ng/ml TPA resulted in a 10 fold activation over the basal level of SEAP expression (Fig. 4). Stimulation of the cells with 140 μM (40 μg/ml) nidulal induced the AP-1 mediated SEAP-expression 2.5 fold as compared to the uninduced control. In contrast to the AP-1 mediated SEAP expression, the NF-κB, CREB and glucocorticoid receptor dependent SEAP expression was not influenced by the addition of nidulal (Fig. 4). These results indicate a first hint for a more selective activity of (**1**).

In order to determine the effect of (**1**) on activation of the transcription factor complex AP-1, EMSA's with whole cell extracts have been performed with a high affinity binding site.

In nonstimulated, proliferating HL-60 cells a strong AP-1 binding activity could be observed (control, Fig. 5, band 1), which disappeared after addition of a 10 fold excess of unlabelled AP-1 probe. Stimulation of HL-60 cells with TPA, an activator of protein kinase C, for 16 hours resulted in an activation of the upper AP-1 binding activity (band 1) twofold over the basal level in non-

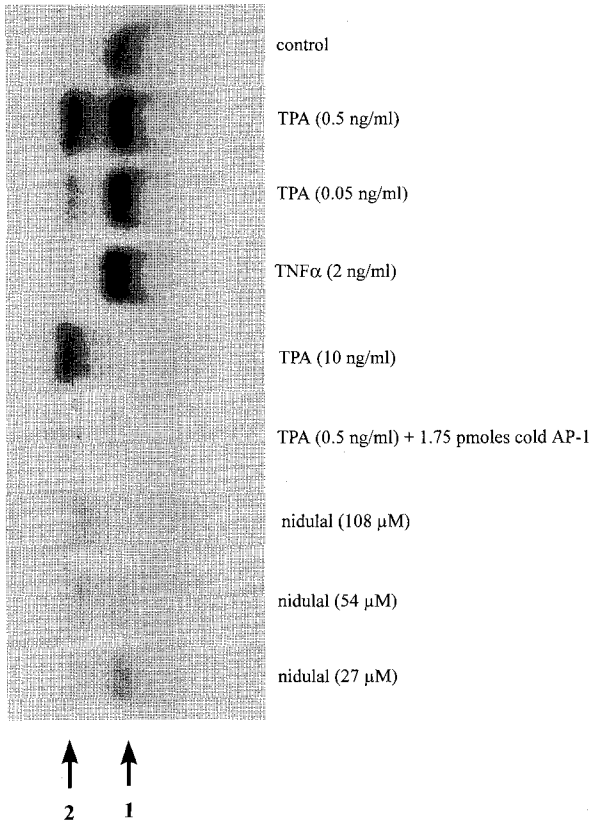
Fig. 4. Effect of nidulal (**1**) and niduloic acid (**2**) on SEAP expression in COS-7 cells.



COS-7 cells have been transiently transfected with various SAEP reporter constructs and induced with 50 ng/ml TPA (pGE2-AP1, pGE2-NF-κB), 300 μg/ml Cl-cAMP and 500 μM IBMX (pGE2-CRE), 1 μM dexamethasone (pGE2-GRE) as control or nidulal (140 μM) and niduloic acid (144 μM). The value for unstimulated vector transfected control was taken as 1.

stimulated cells and also in an alteration of the composition of the AP-1 transcription factor complex. Upon induction with 0.5 ng/ml TPA, the appearence of a second faster migrating band (band 2) could be observed whereas 10 ng/ml TPA resulted in a complete loss of the upper band. The appearence of the two bands may be explained by the formation of either cJun : cJun homodimers or

Fig. 5. Effect of nidulal on the activation of AP-1 in HL-60 cells.



HL-60 cells were treated for 16 hours with various concentrations of TPA, TNF (2 ng/ml) or nidulal. Total cell extracts of equal amounts of protein were then analyzed by EMSA for DNA binding activity of AP-1 using a ^{32}P-labelled oligonucleotide with the high affinity site 5′-CGCTTGATGAGTCAGCCGGAA-3′. The inducible AP-1 complexes are indicated by arrows. A section of the fluorogram from a native gel is shown.

cJun : cFos heterodimers[12]. After treatment of the cells with 2 ng/ml TNF-α, which induces differentiation, only a twofold increase of the upper AP-1 binding activity could be observed (measured by densiometry scanning). Preincubation of HL-60 cells with different concentrations of nidulal resulted in a reduction of the total AP-1 binding activity (band 1, 2), which may be due to the proliferation inhibiting activity of the compound. However the alterations in the composition of the AP-1 complexes after treatment with nidulal were similar to the alteration in DNA binding activity caused by TPA. Similar effects on AP-1 activation and differentiation in U-937 cells after induction by TPA have recently described by WAYS *et al.*[13].

Other Biological Activities

Nidulal exhibits weak antibiotic activities. In the serial dilution assay the MICs for *Salmonella typhimurium* TA98, *Nematospora coryli* and *Ustilago nuda* were determined to 72 ~ 181 μM and 181 ~ 362 μM for *Nematospora coryli* and *Penicillium notatum*.

In the test for mutagenicity according to AMES *et al.* no induction of revertants of *S. typhimurium* TA98 and TA 100 could be observed with 100 μg of nidulal/plate (pour plate assay with and without addition of rat liver microsomes).

Niduloic acid (**2**) induces the differentiation of 15 ~ 25% of HL-60 cells at a concentration of 36 μM. An inhibition of proliferation of HL-60 cells could be observed at concentrations ranging from 72 μM up to 144 μM. In the reporter gene assay with COS-7 cells (**2**) increased the AP-1 dependent SEAP expression 3 fold at a concentration of 144 μM. Niduloic acid showed also weak antibiotic activity against the yeast *Nematospora coryli*.

Discussion

Nidulal (**1**) is a new bisabolane sesquiterpene which induces the differentiation of HL-60 and U-937 cells. The differentiation of HL-60 cells is followed by apoptosis. Both events are regulated by similar signal transduction networks ending with the activation of transcription factors and transcription of the corresponding genes[14]. The same transcription factor may be activated by different signal transduction pathways. In COS-7 cells nidulal (**1**) selectively activates AP-1 dependent signal transduction in a manner similar to TPA. In the gel shift assay a change of binding activities is observed when HL-60 cells are induced by nidulal (**1**). This may be the result of a changing composition of the proteins of the AP-1 complex (cJun : cJun or cJun : cFos) during differentiation. Similar effects on AP-1 activation are observed with TPA, which is a strong inducer of HL-60 cell differentiation. AP-1 activation has been reported to be important for TPA induced differentiation in HL-60 and U-937 cells by HAAS[15].

Acknowledgments

Financial support from the Deutsche Forschungsgemeinschaft and the Swedish Science Research Council is gratefully acknowledged.

References

1) COLLINS, S. J.; R. C. GALLO & R. E. GALLAGHER: Continuous growth and differentiation of human myeloid leukemia cells in suspension culture. Nature 270: 347 ~ 349, 1977
2) MCGAHON, A. J.; J. M. SEAMUS, R. P. BISSONNETTE, A. MAHBOUBI, Y. SHI, R. J. MOGIL, W. K. NISHIOKA & D. R. GREEN: The end of the (cell) line: Methods for the study of apoptosis *in vitro*. Methods in Cell Biology 46: 153 ~ 185, 1995

3) THOMPSON, C. B.: Apoptosis in the pathogenesis and treatment of disease. Science 267: 1456~1462, 1995
4) BRODIE, H. J.: The Birds Nest Fungi. University of Toronto Press, Toronto, 1975
5) ANKE, H.; O. BERGENDORFF & O. STERNER: Assays of the biological activities of guaiane sesquiterpenes isolated from fruit bodies of edible Lactarius species. Food Chem. Toxic. 27: 393~397, 1989
6) ERKEL, G.: Neue Inhibitoren der AMV-Reverse Transkriptase aus Basidiomyceten. Ph. D. Thesis, Univ. Kaiserslautern, 1990
7) AMES, B.; J. MCCANN & E. YAMASAKI: Methods for detecting carcinogenes and mutagenes with the Salmonella/Mammalian mutagenicity test. Mutat. Res. 31: 347~364, 1975
8) ANKE, T.; G. ERKEL, G. KOCKSCH, A. KUSCHEL, A. GIMENEZ, R. VELTEN & W. STEGLICH: Inhibitoren der HIV-1-Reversen Transkriptase und Induktoren der Differenzierung Menschlicher Zellinien aus Pilzen. In Wege zu Neuen Produkten und Verfahren der Biotechnologie. DECHEMA Monographien. 129, pp. 15~25, VCH, Stuttgart, 1993
9) NAGY, L.; V. A. THOMAZY, G. L. SHIPLEY, L. FESUS, W. LAMPH, R. A. HEYMAN, R. A. S. CHANDRARATNA & P. J. A. DAVIES: Activation of retinoid X receptors induces apoptosis in HL-60 cell lines. Mol. Cell. Biol. 15: 3540~3551, 1995
10) ERKEL, G.; T. ANKE & O. STERNER: Inhibition of NF-κB activation by panepoxydone. Biochem. Biophys. Res. 226: 214~221, 1996
11) PAHL, H. L. & P. BAUERLE: A novel signal transduction pathway from the endoplasmatic reticulum to the nucleus is mediated by transcription factor NF-κB. The EMBO J. 14: 2580~2588, 1995
12) ANGEL, P. & M. KARIN: The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. Biochem. Biophys. Acta 1072: 129~157, 1991
13) WAYS, D. K.; W. QIN, T. O. GARRIS, J. CHEN, E. HAO, D. R. COOPER, S. J. USALA, T. J., PARKER & P. P. COOK: Effects of chronic phorbolester treatment on protein kinase C activity, content, and gene expression in the human monoblastoid U 937 cell. Cell growth and Differentiation 5: 161~169, 1994
14) HALE, A. J.; C. A. SMITH, L. C. SUTHERLAND, V. E. A. STONEMAN, V. L. LONGTHORNE, A. C. ULHANE & G. T. WILLIAMS: Apoptosis: molecular regulation of cell death. Eur. J. Biochem. 236: 1~26, 1996
15) HASS, R.: Retrodifferentiation - an alternative biological pathway in human leukemia cells. Europ. J. Cell Biol. 58: 1~11, 1992