PERGAMON

Phytochemistry 53 (2000) 257–263

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Study of sesquiterpene lactones from *Milleria quinqueflora* on their anti-inflammatory activity using the transcription factor NF-κB as molecular target ☆

V. Castro [a], P. Rüngeler [b], R. Murillo [a], E. Hernandez [a], G. Mora [c], H.L. Pahl [d], I. Merfort [b,*]

[a] *Universidad de Costa Rica, Escuela de Química and CIPRONA, San José, Costa Rica*
[b] *Institute of Pharmaceutical Biology, Albert-Ludwigs-Universität, Schänzlestr. 1, 79104, Freiburg, Germany*
[c] *Universidad de Costa Rica, Faculdad de Farmacia and CIPRONA, San José, Costa Rica*
[d] *Department of Experimental Anaesthesiology, University Hospital, Freiburg, Germany*

Received 14 April 1999; received in revised form 24 June 1999; accepted 13 September 1999

Abstract

In Central America aerial parts of the Asteraceae *Milleria quinqueflora* are used in traditional medicine as a remedy for skin infections. Reinvestigation of this plant afforded thirteen sesquiterpene lactones (Sls), three of them are new. All isolated Sls were studied for their anti-inflammatory activity using the transcription factor NF-κB as molecular target. NF-κB is involved in the synthesis of inflammatory mediators, such as cytokines and chemokines. NF-κB DNA binding was inhibited at micromolar concentrations by all Sls.

Keywords: *Milleria quinqueflora*; Asteraceae; Sesquiterpene lactones; Anti-inflammatory activity; NF-κB

1. Introduction

In Central America aerial parts of the Asteraceae *Milleria quinqueflora* L. are used in traditional medicine as a remedy for skin infections (Heinrich, 1996). Previously several sesquiterpene lactones (Sls) from the germacranolide type (melampolides and millerenolides) have been isolated (Jakupovic, Castro & Bohlmann, 1987). Few of them were investigated for their anti-inflammatory activity using the transcription factor NF-κB as molecular target. It was shown that they were able to inhibit DNA binding of NF-κB in micromolar concentrations (Rüngeler et al., 1999). NF-κB, a central mediator of the human immune response, regulates the transcription of genes encoding various inflammatory cytokines, chemokines, adhesion molecules and inflammatory enzymes like iNOS, COX-2, 5-LOX and cytosolic phospholipase A_2 (Baeuerle & Henkel, 1994; Barnes & Karin, 1997).

In this paper, we report on the reinvestigation of the aerial parts of *M. quinqueflora* (see Fig. 1). Altogether, thirteeen Sls were isolated and identified, three are reported for the first time. All compounds were studied for their ability to inhibit the transcription factor NF-κB. To gain information, whether NF-κB inhibition may correlate with the lipophilicity of the Sls, we additionally determined the octanol/water partition coefficient (log *P*) of each compound, from which penetration behavior through biomembranes may be deduced.

2. Results and discussion

The lipophilic extract prepared from the aerial parts

☆ Part III in the series 'Phytochemical and Biological Studies of Costa Rican Asteraceae'.

* Corresponding author. Tel.: +49-761-203-2804; fax: +49-761-203-2803.

E-mail address: merfort@sun2.ruf.uni-freiburg.de (I. Merfort).

0031-9422/00/$ - see front matter
PII: S0031-9422(99)00510-5

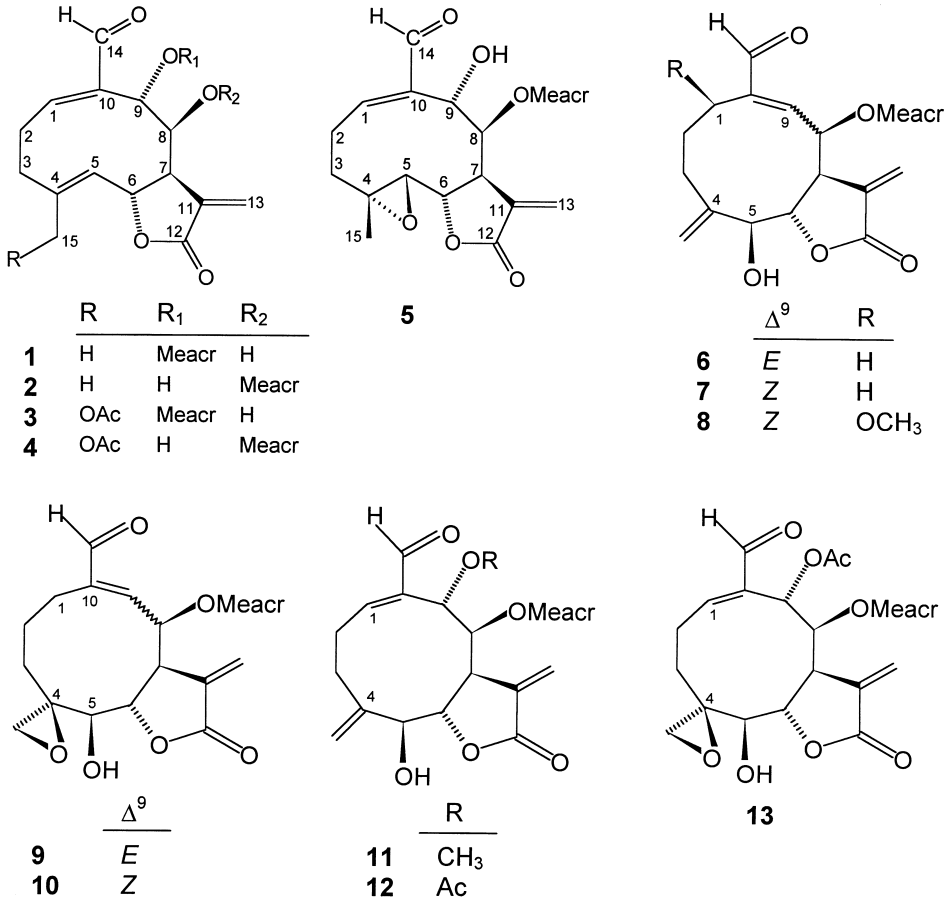

Fig. 1. Structures of the investigated sesquiterpene lactones (Meacr = methacryloyl, *E* = trans, *Z* = cis).

of *M. quinqueflora* afforded, after chromatography, thirteen germacranolide-type Sls. Eight of them, compounds **2**–**7**, **9** and **10**, are already known from this plant (Jakupovic et al., 1987). Since the ^{13}C-NMR spectral data of Sls **2**, **4**, **5** and **7** had not been previously reported, they are included in Table 1.

Compound **1** possesses a molecular mass of 346 (CI(isobutane) and ESI MS). The ^{13}C-and ^{1}H-NMR data were close to those of 9α-hydroxy-8ß-methacryloyloxy-14-oxo-acanthospermolide (**2**) (see Tables 1 and 2) (Jakupovic et al., 1987). Only the chemical shifts for the protons eight and nine as well as those for the carbons one, eight, nine and ten showed significant differences. Therefore, it can be concluded that compounds **1** and **2** are isomers, differing only in the side of esterification. Consequently, Sl **1** is 8ß-hydroxy-9α-methacryloyloxy-14-oxo-acanthospermolide. All proton and carbon resonances were assigned on the basis of ^{1}H–^{1}H COSY, DEPT, GHSQCR and GHMQCR experiments (Tables 1 and 2).

The ^{13}H-NMR spectrum of Sl **8**, from which the molecular mass of 376 was deduced from the CI (isobutane) and ESI mass spectra, was very close to that of 1ß-ethoxymiller-9*Z*-enolide (Jakupovic et al., 1987). The only difference was the additional singlet for a methoxy group at δ 3.25 ppm and the lack of the signals for the ethoxy group. Thus, **8** was 1ß- methoxymiller-9*Z*-enolide, which is an artefact arising during extraction with methanol. This was proved by extracting a small amount of the plant material with ethanol. In this extract compound **8** could not be detected.

The ^{1}H-NMR data of **11** were similar to those reported for the millerenolide miller-1(10)*Z*-enolide (Jakupovic et al., 1987). However, the molecular mass which was deduced from the mass spectra (CI (isobutane) and ESI) increased to 376 due to the presence of an additional methoxy group which is confirmed by the singlet at δ 3.18 in the ^{1}H-NMR spectrum. The methoxy group was located at carbon nine. This was deduced from the downfield shifts of H-9 and H-8 compared to the unsubstituted miller-1(10)*Z*-enolide and the long range H–C correlations observed in the GHMQCR spectrum between the signal of the methoxy group and that of C-9. According to the large coupling constant $J_{8,9} = 9.3$ Hz the methoxy group was α-orientated. Assignments of the protons and car-

Table 1
^{13}C-NMR data of sesquiterpene lactones from *M. quinqueflora* (75*/100 MHz, $CDCl_3$, TMS as internal standard, multiplicities by DEPT)

C	**1***	**2**	**4**	**5**	**7**	**11***	**12**	**13**
1	159.3 *d*	155.2	154.5 *d*	156.1	32.6	155.1 *d*	157.8	158.1
2	26.9 *t*	26.5	27.2 *t*	25.6	21.5	26.7 *t*	27.5	27.2
3	37.0 *t*	36.9	33.1 *t*	35.8	26.0	29.7 *t*	29.5	29.0
4	138.3 *s*	137.4	135.8 *s*	59.4	144.4	143.0 *s*	143.6	57.3
5	127.0 *d*	126.7	131.7 *d*	63.3	81.8[a]	80.2[a] *d*	80.4[a]	77.3[a]
6	74.3 *d*	75.1	73.6 *d*	76.0	79.8[a]	80.3[a] *d*	80.5[a]	78.1[a]
7	51.4 *d*	51.4	51.4 *d*	46.3	44.6	41.1 *d*	41.3	40.4
8	68.0 *d*	72.0[a]	72.1 *d*	72.8	71.7	75.3 *d*	74.4	74.5
9	73.3 *d*	70.7[a]	70.4 *d*	70.8	142.8	78.0 *d*	69.0	69.0
10	141.4 *s*	144.4	144.5 *s*	143.9	137.2	137.9 *s*	137.4	135.3
11	135.7 *s*	134.2	135.3 *s*	133.4	133.3	133.6 *s*	133.7	133.4
12	169.6 *s*	169.0	168.7 *s*	168.1	168.6	168.6 *s*	168.5	168.6
13	120.3 *t*	121.5	122.0 *t*	123.2	125.5	126.2 *t*	126.7	126.6
14	194.1 *d*	195.0	195.3 *d*	194.8	190.3	191.8 *d*	191.9	192.0
15	18.2 *q*	17.0	61.3 *t*	18.1	117.0	121.6 *t*	121.6	54.3
1′	166.8 *s*	166.9	167.1 *s*	167.1	166.1	165.8 *s*	165.7	165.4
2′	135.8 *s*	135.3	133.8 *s*	135.4	135.0	135.5 *s*	135.2	134.8
3′	126.7 *t*	126.7	127.0 *t*	127.3	127.1	126.5 *t*	127.0	126.6
4′	17.0 *q*	18.5	18.3 *q*	18.8	18.2	18.3 *q*	18.6	18.3
1″			170.4 *s*				170.4	170.3
2″			20.7 *q*				21.0	20.8
OCH_3						57.6 *q*		

[a] assignments may be interchanged.

bons followed from the $^{-1}$H-^{1}H COSY, DEPT, and GHMQCR spectra (see Tables 1 and 2). Thus, Sl **11** is 9α-methoxy-miller-1(10)*Z*-enolide, which again is an artefact not detectable in the ethanolic extract.

Structure elucidation based on MS (CIMS) and NMR (^{1}H-NMR, ^{1}H–^{1}H COSY, ^{13}C-NMR, DEPT, HMBC and NOESY) analysis led to the identfication of **12** as 9α-acetoxy-miller-1(10)*Z*-enolide. On the basis of the great similarities between the ^{1}H and ^{13}C-NMR spectral data of compounds **11** and **12**, the stereoche-

Table 2
^{1}H-NMR data of sesquiterpene lactones from *M. quinqueflora* (300*/400 MHz, $CDCl_3$, TMS as internal standard)

H	**1***		**8***		**11***		**12**		**13**	
	δ	*J* (Hz)	δ	*J*(Hz)	δ	*J*(Hz)	δ	*J*(Hz)	δ	*J* (Hz)
1	6.73 *dd*	7.6, 10.0	4.34 *m*		6.69 *dd*	5, 12.9	6.66 *dd*	5, 13	6.87 *dd*	5, 13
2α	2.93 *dddd*	2.7, 10.0, 12.5, 14.9	2.00–2.20[a]		2.58 *m*		2.62 *dddd*	4, 5, 5, 13	2.60 *dddd*	2, 4, 5, 15
2β	2.62 *dddd*	2.0, 2.2, 7.6, 14.9			2.80 *m*		2.91 *dddd*	5, 12, 13, 13	3.09 *dddd*	3.5, 13, 14, 15
3α	2.08 *m*		1.80–2.00[a]		2.38 *m*		2.38 *ddd*	5, 12, 13	1.46 *ddd*	2, 14, 15
3β	2.42 *m*				3.07 *m*		3.10 *ddd*	4, 5, 13	2.94 *ddd*	3.5, 4, 15
5	4.88 *d*(*br*)	11.2	3.93 *d*(*br*)	9.5	3.88 *d*(*br*)	9.8	3.91 *d*	9.5	2.79 *d*	9.5
6	5.20 *dd*	10.0, 10.0	4.75 *dd*	4, 9.3	4.73 *dd*	1, 9.5	4.82 *dd*	1, 9.5	5.05 *d*(*br*)	9.5
7	2.42 *m*		3.20 *m*		2.81 *m*		2.83 *m*		3.41 *m*	
8	5.34 *dd*	1.7, 8.3	6.34–6.40[a]		5.95 *d*(*br*)	7.6	6.08 *dd*	2, 10	6.04 *s*(*br*)	
9	5.12 *dd*	2.2, 8.3	6.34–6.40[a]		4.23 *d*(*br*)	9.3	5.94 *dd*	1, 10	6.04 *s*(*br*)	
13a	6.34 *d*	3.7	5.83 *d*	2.2	5.79 *d*	1.7	5.82 *d*	1.5	5.81 *d*	1.5
13b	5.64 *d*	3.2	6.44 *d*	2.7	6.30 *d*	1.5	6.32 *d*	1.5	6.23 *d*	1.5
14	9.41 *d*	2.2	9.75 *s*(*br*)		9.50 *s*(*br*)		9.43 *s*(*br*)		9.50 *s*(*br*)	
15a	1.98 *s*		4.85 *d*(*br*)	2.2	4.95 *s*(*br*)		4.96 *s*(*br*)		2.44 *d*	4
15b			4.99 *d*(*br*)	2.2	5.01 *s*(*br*)		5.01 *s*(*br*)		2.50 *d*	4
$OC\underline{H_3}$			3.25 *s*		3.18 *s*					
3′a	6.11 *s*(*br*)		6.06 *s*(*br*)		6.05 *s*(*br*)		6.03 *s*(*br*)		6.01 *s*(*br*)	
3′b	5.60 *dq*	1.2	5.60 *dq*	1.2	5.54 *dq*	1.3	5.54 *s*(*br*)		5.54 *s*(*br*)	
4′CH_3	1.91 *s*(*br*)		1.88 *dd*	1, 1.5	1.84 *s*(*br*)		1.84 *s*(*br*)		1.84 *s*(*br*)	
$C\underline{H_3}$–Ac							1.94 *s*		1.94 *s*	

[a] Overlapping signals.

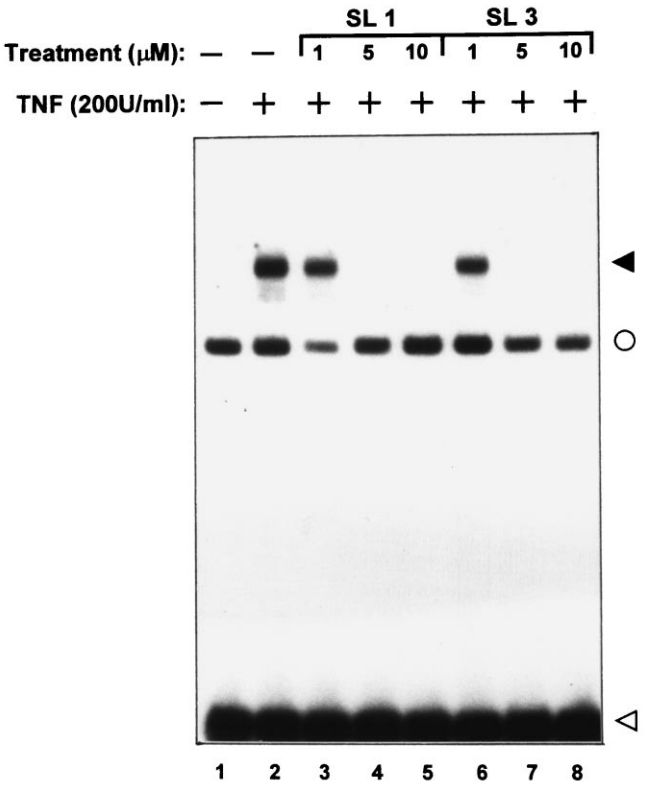

Fig. 2. The effect of the sesquiterpene lactones **1** and **3** on NF-κB DNA binding. Lane 1 shows unstimulated control cells, in lane 2 cells were treated with 200 U/ml TNF-α alone. In lanes 3–8 cells were pretreated for one hour with various concentrations of compound **1** and **3** and subsequently stimulated with TNF-α for 1 h. A filled arrowhead indicates the position of NF-κB DNA complexes. The open circle denotes a non-specific activity binding to the probe. The open arrowhead shows unbound oligonucleotide.

mical structure of both must be the same. Only the signal for the methoxy group was replaced by a three-proton singlet at δ 1.94 of an acetyl group. Esterification of C-9-OH with acetic acid and of C-8-OH with methacrylic acid was deduced from the HMBC spectrum, because of the cross peak arising from the carbonyls of the respective acids ($\delta = 170.4$ and 165.7) and H-9 or H-8. The structure of compound **13** followed from its mass spectral and NMR (^{1}H-NMR, ^{1}H–^{1}H COSY, ^{13}C-NMR and NOESY) data. The ^{13}C-NMR data markedly differed from those of **12** in the chemical shifts for C-4 and C-15, slightly in the signals for C-5 and 6 (see Table 1). This is in accordance with the replacement of the exocylic methylene group in compound **12** by an epoxy function at C-4 in **13**. Two one-proton doublets at δ 2.44 and 2.50 in the ^{1}H-NMR spectrum confirmed this structure element. The stereochemistry at C-4 was elucidated from the dipolar interaction (NOE) between H-15 and H-7. Thus, compound **13** which possesses the molecular mass of 420 was identified as 9α-acetoxy-4ß,15-epoxy-miller-1(10)*Z*-enolide.

Table 3
Results of the NF-KB assay and log *P* values

SL	Inhibitory concentartion (μM)	log *P*
1	5	2.0
2	10	1.9
3	5	1.9
4	5	1.8
5	10	1.6
6	5	2.0
7	10	1.9
8	20	2.0
9	5	1.9
10	5	1.8
11	50	1.4
12	10	1.7
13	5	1.7

In order to investigate whether the Sls **1**–**13** interfere with the activation of transcription factor NF-κB, Jurkat T cells were incubated with the respective Sl at various concentrations for one hour and subsequently stimulated with TNF-α for one hour. Total protein extracts were prepared and analysed for NF-κB DNA binding activity in an electrophoretic mobility shift assay (EMSA) (see Fig. 2). Stimulation with TNF-α induced one novel DNA binding activity in Jurkat T cells (Fig. 2, lane 2). Antibody reactivity and competition assays identified this complex as an NF-κB p50/p65 heterodimer (data not shown; see Pahl & Baeuerle, 1995; Brown, Park, Kanno, Franzoso & Siebenlist, 1993). Despite Sls **8** and **11** are not natural products, but artefacts they were investigated in order to find out whether the space filling methoxy group close to the α,ß-unsaturated aldehyde group and also its position may influence the inhibitory activity.

Except for compounds **8** and **11** all Sls completely inhibited NF-κB DNA binding at a concentration of 5 or 10 μM without exhibiting cytotoxic effects (see Table 3). Neither the conformation of the basic carbocyclic skeleton (in Fig. 3, as examples, the calculated conformations with the lowest energy from the new Sls **1**, **8** and **11** are given) nor the introduction of a further exocyclic methylene group at carbon four or an epoxide significantly influenced the inhibitory activity. Only the introduction of a space filling methoxy group near the aldehyde group decreased the inhibitory activity so that 20 (Sl **8**) or 50 μM (Sl **11**) are required. Furthermore, also the position of the methoxy group had an influence on the inhibitory concentration.

We also investigated the impact of lipophilicity on inhibitory activity by determining the logarithmic partition coefficient log *P* by an HPLC-method according OECD guidelines. All log *P* values were in a narrow range between 1.4 and 2.0 (see Table 3). The most hydrophilic compound **11** (log *P* 1.4) possesses the lowest inhibitory activity. However, regarding our recent

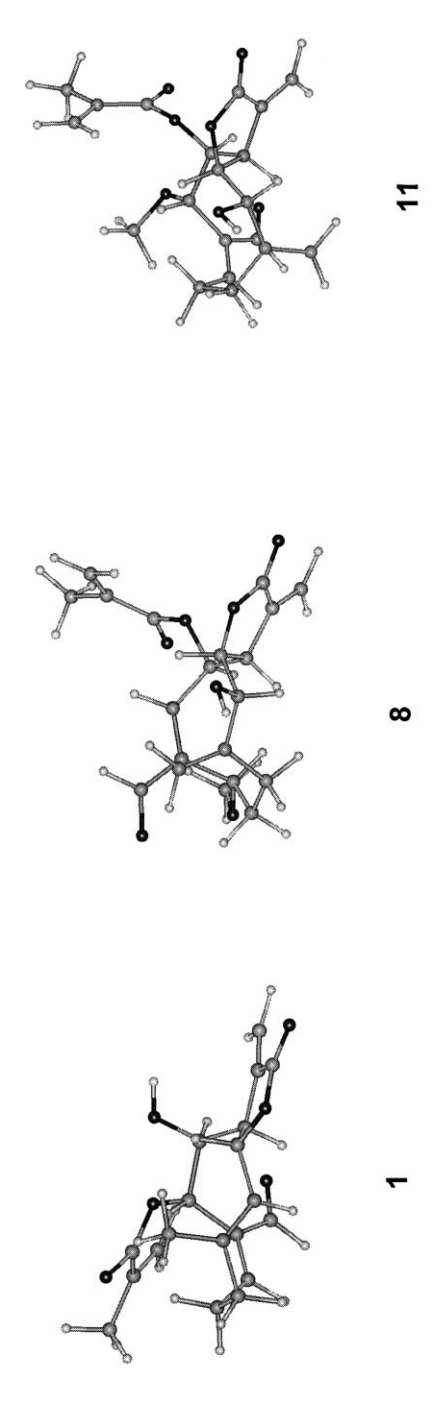

Fig. 3. Calculated conformations of sesquiterpene lactones **1**, **8** and **11**.

studies which showed that Sls with a similar log *P* exhibit significant differences in their ability to inhibit NF-κB it is unlikely that lipophilicity influences NF-κB inhibitory activity to a large degree.

Our results with the bifunctional Sls from *M. quinqueflora* are in accordance with our recent studies (Lyß, Knorre, Schmidt, Pahl & Merfort, 1998; Rüngeler et al., 1999). Here, we provided evidence that Sls selectively alkylate the p65 subunit of NF-κB thus preventing its DNA binding. The number of alkylating centers of the Sls plays the most important role in determining a strong NF-κB inhibitory activity. Sls which possess two reactive centres in form of an α-methylene-γ-lactone group and an α,ß- or α,ß,γ,δ-unsaturated carbonyl group are the most active ones. Based on computer molecular modelling, we proposed a molecular mechanism of action how these bifunctional Sls may alkylate the p65 subunit of NF-κB. They can crosslink two cysteine residues, Cys 38 being located within the DNA binding domain and Cys 120 being found in a proximal loop, thereby inhibiting DNA binding (Chen, Ghosh & Ghosh, 1998).

In summary, our study shows that the isolated Sls possess a high anti-inflammatory activity by inhibiting the transcription factor NF-κB at micromolar concentrations. Thereby they are able to contribute to the healing effects of preparations from *M. quinqueflora* used in traditional medicine for the treatment of skin infections. Furthermore, Sls could serve as lead compounds for the development of novel, potent anti-inflammatory drugs for the treatment of inflammatory disorders such as rheumatoid arthritis.

3. Experimental

3.1. List of abbreviations

BSA: bovine serum albumen; c.p.m.: counts per minute; DTT: dithiothreitol; EDTA: ethylenediaminetetraacetic acid; EGTA: ethylene glycol-bis(β-aminoethyl ether) *N*,*N*,*N*′;*N*′ tetraacetic acid; HEPES: (*N*-[2-hydroxyethyl] piperazine-*N*′-[2-ethanesulfonic acid]); NP-40: (nonylphenoxy)-(polyethoxy)-ethanol; PMSF: phenylmethylsulfonyl fluoride; Poly (dI-dC): polydeoxyinosinicdeoxycytidylic acid, double-stranded alternating copolymer.

3.2. Plant material

Aerial parts of *M. quinqueflora* L. were collected in Turrialba, Costa Rica, in February 1998 and identified by L. Poveda, Professor of Botany, Universidad Nacional, Costa Rica. Voucher specimens (no. 108510a) are deposited at the National Herbarium of Costa Rica.

3.3. Cell culture

Jurkat T cells were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum and 100 IU/ml penicillin and 100 μg/ml streptomycin (all Gibco-BRL).

3.4. Electrophoretic mobility shift assays

Total protein extracts from Jurkat T cells were prepared using a high-salt detergent buffer (Totex: 20 mM Hepes, pH 7.9, 350 mM NaCl, 20% (v/v) glycerol, 1% (w/v) NP-40, 1 mM $MgCl_2$, 0.5 mM EDTA, 0.1 mM EGTA, 0.5 mM DTT, 0.1% PMSF, 1% aprotinin). Cells were harvested by centrifugation, washed once in icecold PBS (Sigma) and resuspended in four cell volumes of Totex buffer. The cell lysate was incubated on ice for 30 min, then centrifuged for 5 min at 13,000 rpm at 4°C. The protein content of the supernatant was determined and equal amounts of protein (10–20 μg) added to a reaction mixture containing 20 μg bovine serum albumen, BSA, (Sigma), 2 μg poly (dI-dC) (Boehringer), 2 μl buffer D+ (20 mM Hepes, pH 7.9; 20% glycerol, 100 mM KCl, 0.5 mM EDTA, 0.25% NP-40, 2 mM DTT, 0.1% PMSF), 4 μl buffer F (20% Ficoll 400, 100 mM Hepes, 300 mM KCl, 10 mM DTT, 0.1% PMSF) and 100000 c.p.m. (Cerenkov) of a ^{32}P-labeled oligonucleotide, made up to a final volume of 20 μl with destilled water. Samples were incubated at room temperature for 25 min. NF-κB oligonucleotide (Promega) was labeled using γ-[^{32}P]-ATP (3000 Ci/mmol; Amersham) and a T4 polynucleotide kinase (Promega).

3.5. Partition coefficient (n-octanol/water); HPLC-method

The partition coefficients of the different compounds were determined using an HPLC method following the OECD guideline for testing of chemicals No. 117 (1989) (adopted 30.03.1989). HPLC was performed on an analytical RP-18 column (Bischoff ODS-Hypersil 5 μm; 125 × 4.6 mm) with mixtures of HPLC-grade methanol and distilled water (30%, 40% or 50% methanol). A diode array detector was used. The dead time t_0 was measured with thiourea (Fluka), which leaves the column unretained. As reference compounds we used 2-butanone (log *P*: 0.3; Merck), benzyl alcohol (log *P*: 1.1; Roth), benzonitrile (log *P*: 1.6; Fluka), acetophenone (log *P*: 1.7; Fluka), cinnamic alcohol (log *P*: 1.9; Roth), anisole (log *P*: 2.1; Merck), methyl benzoate (log *P*: 2,1; Fluka), 1,1,1-trichloroethane (log *P*: 2,4; Fluka), ethyl benzoate (log *P*: 2.6; Fluka), allyl phenyl ether (log *P*: 2.9; Fluka), thymol (log *P*: 3.3; Merck) and naphthalene (log *P*: 3.6; Aldrich). The partition coefficient is deduced from the capacity factor *k*, which is derived from the following formula:

$$k = \frac{t_R - t_0}{t_0}$$

where t_R is the retention time of the substance. The logarithms of the capacity factors of the reference compounds, log *k*, are calculated and plotted as a function of log *P*. The log *P* of Sls is obtained by measuring the retention times and interpolating the calculated capacity factors on the calibration graph. For each compound retention times were measured twice. The calibration was performed once daily.

3.6. Calculation of the conformations for Sls **1**, **8** and **11**

We generated low-energy conformations of the Sls using the conformational search option of ChemPlus® (v. 1.6), which is operated under the Molecular Modelling package Hyperchem® (v. 5.1). Energy minimizations were performed with Hyperchem's force field MM+ using the Polak-Ribiere minimization algorithm. Starting structures were created with Hyperchem and initially minimized to an RMS gradient < 0.01 kcal mol^{-1} Å^{-1}. All rotatable cyclic bonds were included as variable torsions and allowed to be changed simultaneously. The search was performed applying a usage directed search method and standard settings for duplication tests. A search run was terminated after energy minimization of 2500 unique starting geometries. Acyl side chains of the respective Sls were not included in the conformational search. They were added manually to the most favorable Sl-conformers. The resulting structures were energy minimized to an RMS gradient as above.

3.7. Extraction and isolation

Aerial parts of *M. quinqueflora* (3600 g) were air dried, powdered and extracted with ether. The ether extract was defatted with MeOH and separated as previously described (Jakupovic et al., 1987). The following amounts of the respective compounds were obtained: **1**: 34 mg, **2**: 20 mg, **3**: 17 mg, **4**: 84 mg, **5**: 6 mg, **6**: 47 mg, **7**: 7 mg, **8**: 6 mg, **9**: 250 mg, **10**: 480 mg, **11**: 13 mg, **12**: 140 mg, **13**: 73 mg.

3.7.1. 8ß-Hydroxy-9α-methacryloyloxy-14-oxo-acanthospermolide (**1**)

CIMS (*iso*-butane) *m/z* (rel. int.): 347 $[M + H]^+$ (46), 261 $[M - C_4O_2H_6 + H]^+$ (100), 243 $[261 - H_2O]^+$ (83), 233 (29), 217 (46), 201 (31), 177 (40), 87 $[C_4O_2H_6 + H]^+$ (93). ESIMS *m/z* (rel. int.): 715 $[2 \times M + Na]^+$ (19), 401 $[M + 54 + H]^+$ (51), 385 $[M + K]^+$ (33), 369 $[M + Na]^+$ (100), 347 $[M + H]^+$ (10), 261 (13).

*3.7.2. 1ß-Methoxymiller-9Z-enolide (**8**)*

CIMS (*iso*-butane) *m/z* (rel. int.): 377 $[M + H]^+$ (3), 291 $[M - C_4O_2H_6 + H]^+$ (9), 267 (30), 197 (100), 87 $[C_4O_2H_6 + H]^+$ (97). ESIMS *m/z* (rel. int.): 776 $[2 \times M + Na + H]^+$ (5), 431 $[M + 54 + H]^+$ (51), 415 $[M + K]^+$ (33), 399 $[M + Na]^+$ (100), 377 $[M + H]^+$ (8), 291 (18).

*3.7.3. 9α-Methoxy-miller-1(10)Z-enolide (**11**)*

CIMS (*iso*-butane) *m/z* (rel. int.): 377 $[M + H]^+$ (30), 291 $[M - C_4O_2H_6 + H]^+$ (58), 275 (18), 261 $[291 - CH_2O]^+$ (35), 247 (24), 243 (20), 231 (20), 217 (25), 215 (33), 87 $[C_4O_2H_6 + H]^+$ (100). ESIMS *m/z* (rel. int.): 776 $[2 \times M + Na + H]^+$ (0.9), 415 $[M + K]^+$ (29), 399 $[M + Na]^+$ (100), 377 $[M + H]^+$ (5), 291 (2).

*3.7.4. 9α-Acetoxy-miller-1(10)Z-enolide (**12**)*

CIMS (*iso*-butane) *m/z* (rel. int.): 405 $[M + H]^+$ (12), 345 $[M - C_2H_4O_2 + H]^+$ (29), 319 $[M - C_4O_2H_6 + H]^+$ (10), 301 (12), 277 (15), 259 (76), 241 (36), 213 (31), 207 (35), 183 (40), 87 $[C_4O_2H_6 + H]^+$ (100), 79 (76).

*3.7.5. 9α-Acetoxy-4ß,15-epoxymiller-1(10)Z-enolide (**13**)*

CIMS (*iso*-butane) *m/z* (rel. int.): 421 $[M + H]^+$ (7), 361 $[M - C_2H_4O_2 + H]^+$ (81), 335 $[M - C_4O_2H_6 + H]^+$ (60), 307 (11), 275 (100), 257 (39), 229 (42), 201 (22), 87 $[C_4O_2H_6 + H]^+$ (78), 79 (63).

Acknowledgements

We are grateful to V. Brecht, Pharmazeutisches Institut, University of Freiburg for recording the 300/75 MHz NMR spectra, to Dr. J. Wörth and C. Warth, Institut für Organische Chemie, University of Freiburg for measuring the mass spectra, to Dr. T.J. Schmidt, Institute of Pharmaceutical Biology, University of Düsseldorf for the help with the calculation of the conformations, to Prof. L. Poveda for identification of the plant material. This work was supported by a grant from the Albert-Ludwigs-Universität and the DAAD (to V.C. and E.H.).

References

Baeuerle, P., & Henkel, T. (1994). *Annu. Rev. Immunol*, *12*, 141.

Barnes, P. J., & Karin, M. (1997). *N. Engl. J. Med*, *336*, 1066.

Brown, K., Park, S., Kanno, T., Franzoso, G., & Siebenlist, U. (1993). *Proc. Natl. Acad. Sci. USA*, *90*, 2532.

Chen, Y.-Q., Ghosh, S., & Ghosh, G. (1998). *Nat. Struct. Biol*, *5*, 76.

Heinrich, M. (1996). In D. J. N. Hind, *Biology and Utilization* (p. 475). In *Proceedings of the International Compositae Conference, Kew, 1994*, *vol. 2*. Kew: Royal Botanic Gardens.

Jakupovic, J., Castro, V., & Bohlmann, F. (1987). *Phytochemistry*, *26*, 2011.

Lyß, G., Knorre, A., Schmidt, T. J., Pahl, H. L., & Merfort, I. (1998). *J. Biol. Chem*, *273*, 33508.

OECD guideline for testing of chemicals No. 117 (adopted 30.03.1989).

Pahl, H. L., & Baeuerle, P. A. (1995). *EMBO J*, *14*, 2580.

Rüngeler, P., Castro, V., Mora, G., Gören, N.,Vichnewski, W., Pahl, H.L., Merfort, I., & Schmidt, T.J. (1999). *Bioorg. Med. Chem*., in press.