Chromogenic Triterpenoids from *Cortinarius fulvoincarnatus*, *C. sodagnitus* and Related Toadstools (Agaricales)

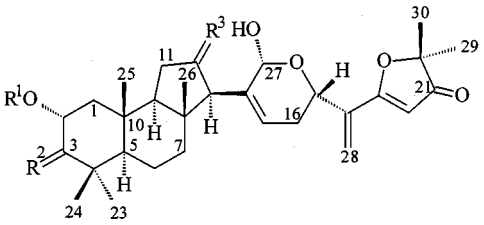
Bernd Sontag,[a] Rita Fröde,[a] Monika Bross,[a] and Wolfgang Steglich*[a]

Dedicated to Professor Günter Adam on the occasion of his 65th birthday

Keywords: Triterpenoids / Sodagnitins / Chromogens / Toadstools / Natural Products

Fruit-bodies of the toadstools *Cortinarius sodagnitus*, *C. fulvoincarnatus*, *C. arcuatorum* (Agaricales) show a remarkable ink-red colour reaction when treated with aqueous base. We isolated six chromogens, named the sodagnitins A–F, from the toadstools and elucidated their structures by spectroscopic techniques. The sodagnitins are triterpenoids of the malabaricane type with a highly modified side chain. A mechanism for the colour reaction is proposed.

Several toadstools of the genus *Cortinarius*, for example *C. arcuatorum* R. Henry, *C.* cf. *calochrous* Fr., *C. fulvoincarnatus* Joach., and *C. sodagnitus* R. Henry show a striking colour reaction.[1,2] When the cap skin or the stalk base of these species is exposed to aqueous alkali an intense inkred tint develops. In the case of *C. fulvoincarnatus* and *C. arcuatorum* the colour reaction can be observed with the whole fruit-body. Unfortunately, all these species are rather rare and the amount of chromogens present in the cap skins is insufficient for a complete structure elucidation. Fortunately, we were able to collect a larger quantity of *C. fulvoincarnatus* that allowed the chemical investigations described in this communication.



	R^1	R^2	R^3
1	H	O	H_2
2	H	O	O
3	HMG	O	H_2
4	HMG	O	O
5	H	H, β-OH	H_2
6	H	H, β-OH	O

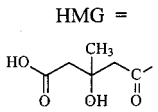


Figure 1. Sodagnitins A−F (**1**−**6**)

Isolation of the Chromogens and Structure Elucidation

The freeze-dried fruit-bodies of *C. fulvoincarnatus* and *C. sodagnitus* were extracted with ethyl acetate, until the colour reaction with alkali was no longer observed. The combined extracts were subjected to gel chromatography followed by purification of the chromogens by preparative HPLC.

The main chromogen of *C. fulvoincarnatus* is sodagnitin C (**3**), which amounts to 0.03% of the fresh weight of the toadstool (Figure 1). Like the other sodagnitins, **3** showed UV maxima (MeOH) at 230 and 292 nm and gave the characteristic red colour reaction with alkali (see below).

In the high resolution atmospheric pressure chemical ionisation mass spectrum (HR-APCIMS) of **3** a $[M^+ + H]$ peak could be observed at *m/z* = 643.3491 corresponding to the molecular formula $C_{36}H_{51}O_{10}$. The molecular weight of 642 is supported by a strong $[M^- - H]$ peak at *m/z* = 641 in the (−)-FAB spectrum and $[M^+ + Na]$ and $[M^+ - H + 2\ Na]$ peaks at *m/z* = 665 and 687, respectively, in the (+)-FAB spectrum. In the IR spectrum (KBr) **3** exhibits strong bands at 1730 (sh, saturated ester), 1720 (carboxyl group, saturated ketone) and 1705 cm^{-1} (unsaturated ketone) and a broad absorption at 3435 cm^{-1} indicating hydroxy functions.

The ^{1}H-NMR spectrum of sodagnitin C (**3**) (Table 1) showed seven clearly separated signals in the olefinic region. The HETCOR spectrum revealed that the signals at δ = 5.74, 5.78, 5.82, and 6.19 belong to olefinic protons, whereas signals at δ = 4.73 (δ_C = 72.5) and 5.68 (δ_C = 64.7) were assigned to two methine groups, each carrying an oxygen atom. The singlet at δ = 5.37 (δ_C = 92.0) was attributed to the CH of a hemiacetal group.

The methine proton resonating at δ = 5.68 is adjacent to the carbonyl group at δ_C = 209.4. In the aliphatic region many signals are coincident and could not be differentiated, even at 600 MHz. Only the signals of seven CH_3 groups at δ = 0.77, 1.11, 1.16, 1.24, 1.40 (2×), and 1.47 could be easily discerned. All appear as singlets and were therefore located on quarternary C atoms. An analysis of the HETCOR spectrum allowed the assignment of the proton

[a] Institut für Organische Chemie der Universität, Karlstrasse 23, D-80333 München, Germany
Fax: (internat.) +49 (0)89/ 5902 604
E-mail: wos@org.chemie.uni-muenchen.de

  1434−193X/99/0101−0255 $ 17.50+.50/0

Table 1. ^{1}H-NMR data of sodagnitin A (**1**), C (**3**), and E (**5**) (600 MHz, in $CDCl_3$)

Position	δ_H **1**	m	*J*	δ_H **3**	m	*J*	δ_H **5**	m	*J*
1	1.19	m		1.51	dd	13, 13	1.50	m	
	2.31	dd	12, 8	2.14	dd	13, 6	2.30	m	
2	4.55	dd	12, 7	5.68	dd	13, 6	3.70	ddd	14, 14, 5
3	---	---		---	---	---	3.00	d	10
5	1.22	m		1.24	m		1.22	m	
6	1.58	m		1.65	m		1.58	m	
	1.67	m							
7	1.32	m		1.36	m		1.32	m	
	1.80	ddd	12, 3, 3	1.80	dd	13, 13	1.88	dd	12, 4
9	1.34	m		1.11	m		1.34	m	
				1.38	m				
11	1.50	m		1.57	m		1.50	m	
	1.63	m					1.63	m	
12	1.76	m		1.76	m		1.76	m	
13	2.30	m		2.32	dd	18, 10	2.28	m	
15	5.75	d	6	5.74	d	5.5	5.76	d	5
16	2.24	ddm	19, 19	2.24	ddd	17, 3, 3	2.22	ddd	17
	2.35	m		2.35	dd	14, 14	2.32	m	
17	4.73	dd	11, 3	4.73	dd	11, 3	4.73	dd	8, 3
20	5.74	s		5.82	s		5.75	s	
23	1.13	s		1.11	s		0.94	s	
24	1.16	s		1.16	s		0.85	s	
25	1.20	s		1.24	s		1.03	s	
26	0.76	s		0.77	s		0.71	s	
27	5.38	s		5.37	s		5.38	s	
28	5.78	s		5.78	s		5.78	s	
	6.18	s		6.19	s		6.18	s	
29	1.41	s		1.40	s		1.41	s	
30	1.41	s		1.40	s		1.41	s	
2′				2.76	m				
4′				2.69	m				
6′				1.47	s				

signals for the remaining CH_2 and CH groups. The coupling constants *J* (Hz) could only be determined in a limited number of cases.

The ^{13}C-NMR spectrum of sodagnitin C (**3**) shows a distribution of 36 signals over a range from δ = 15 to 209 (Table 2). According to the DEPT spectrum the signals belong to 7 CH_3, 9 CH_2 (including one *exo*-methylene group), 8 CH, and 12 quarternary carbon atoms. This result is consistent with the molecular formula $C_{36}H_{50}O_{10}$ derived from the high resolution mass spectrum.

A notable feature is the large number of quarternary carbons, of which the two carbonyl signals at δ = 209.4 and 208.3 could be ascribed to ketone functions, whereas the signals at δ = 172.9 and 170.9 are typical for saturated acids or esters. The signals at δ = 181.7 and 101.2 (CH, δ_H = 5.82) were assigned to the carbons of a donor-acceptor substituted double bond.

A comparison of the mass spectrum of sodagnitin A (**1**) (HR-EI; [M^+]: *m/z* 498.2989, $C_{30}H_{42}O_6$) with that of sodagnitin C (**3**) (HR-APCI; [M^+ + H]: *m/z* = 643.3491, $C_{36}H_{51}O_{10}$) reveals a mass difference of 144 ($C_6H_9O_4$) that is indicative of a 3-hydroxy-3-methylglutaric acid (HMG) residue.

Conjugates of HMG with triterpenoids are known from other basidiomycetes.[3] Finally, a careful analysis of the 2D NMR spectra of sodagnitin C, especially the ^{1}H,^{1}H COSY, NOESY, and HMBC correlations (Figure 2) led to the elucidation of the complete formula.

The structure of the side chain is supported by comparison with the NMR data of known 5,6-dihydro(2*H*)pyran-2-ol and 3(2*H*)furanone derivatives (see below).

The relative configuration of **3** was determined by comparison of the NMR data with the corresponding data published in the literature (see below) and by analysis of the NOE spectra (Figure 3). The all-*trans* configuration of the tricyclic ring system is revealed by the NOE correlations of the β-CH_3 group at C-10 (δ = 1.24) with the H atom at C-2 (δ = 5.68) as well as with the β-CH_3 groups at C-4 and C-8 (δ = 1.16 and 0.77, respectively). The configuration at C-13, C-17, and C-27 is suggested from the observed NOE cross peaks, however, the assignment at C-17 is ambiguous. The absolute configuration of the sodagnitins is as yet unknown.

Sodagnitin C (**3**) was also isolated from *C. arcuatorum* and *C.* cf. *calochrous*, and its presence in some undetermined *Cortinarius* samples exhibiting the red alkali reaction was confirmed by HPLC analysis. The sodagnitins A and C are present in *C. fulvoincarnatus* in relatively high amounts. In contrast, *C. sodagnitus* contains only very low concentrations of the six sodagnitins A−F. After determining the structure of sodagnitin C (**3**) we are able to propose formulae for the minor compounds.

Sodagnitin A (**1**) is derived from sodagnitin C by removal of the HMG residue. The expected changes of the ^{1}H- and ^{13}C-NMR signals were observed at C-1, C-2, and C-3 in

Table 2. ^{13}C-NMR data of sodagnitin A (**1**), C (**3**), and E (**5**) (150 MHz, in $CDCl_3$)

Position	δ_C **1**	m[a]	δ_C **3**	m[a]	δ_C **5**	m[a]
1	49.1	t	45.3	t	46.0	t
2	69.3	d	72.5	d	68.9	d
3	216.2	s	209.4	s	84.2	d
4	47.9	s	49.1	s	39.1	s
5	58.3	d	57.9	d	56.2	d
6	19.9	t	20.1	t	19.2	t
7	40.0	t	40.1	t	40.4	t
8	43.5	s	43.7	s	43.5	s
9	62.4	d	62.6	d	62.9	d
10	37.4	s	37.9	s	37.9	s
11	19.4	t	19.6	t	16.6	t
12	24.8	t	24.9	t	24.8	t
13	52.3	d	52.5	d	52.5	d
14	136.6	s	136.9	s	136.8	s
15	123.3	d	123.4	d	123.1	d
16	30.0	t	30.0	t	30.0	t
17	64.7	d	64.7	d	64.6	d
18	139.1	s	139.1	s	139.1	s
19	181.2	s	181.7	s	181.5	s
20	100.9	d	101.2	d	100.9	d
21	207.6	s	208.3	s	207.6	s
22	87.9	s	88.2	s	87.8	s
23	24.8	q	25.1	q	28.6	q
24	21.0	q	20.8	q	19.3	q
25	16.0	q	16.1	q	16.3	q
26	15.2	q	15.4	q	15.0	q
27	91.9	d	92.0	d	91.9	d
28	121.3	t	121.7	t	121.2	t
29	23.0	q	23.2	q	22.9	q
30	23.0	q	23.2	q	23.0	q
1′			170.9	s		
2′			45.4	t		
3′			70.1	s		
4′			45.3	t		
5′			172.9	s		
6′			27.4	q		

[a] Multiplicities determined by DEPT sequences

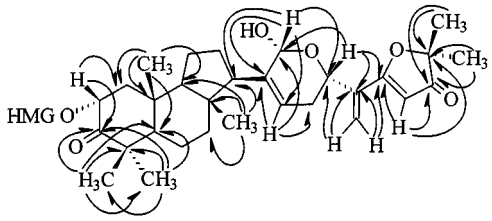


Figure 2. Selected HMBC correlations of sodagnitin C (**3**)

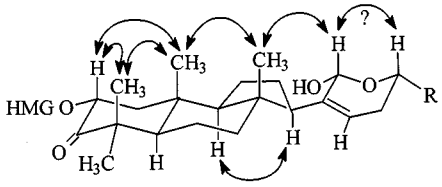


Figure 3. Selected NOE correlations of sodagnitin C (**3**)

agreement with the data reported for analogous cases in the literature.[3,4]

A comparison of the high resolution mass spectrum of sodagnitin A (**1**) (M^+: *m/z* = 498.2989, $C_{30}H_{42}O_6$) with that of sodagnitin E (**5**) (M^+: *m/z* = 500.3138, $C_{30}H_{44}O_6$) indicated that **5** is a dihydro derivative of **1**. An inspection of the ^{1}H- and ^{13}C-NMR spectra revealed the reduction of the carbonyl at C-3 to a β-OH group. The observed NMR shifts are in close accord with those of similar compounds reported in the literature.[5]

The structures of the remaining sodagnitins B (**2**) (M^+: *m/z* = 512.2823, $C_{30}H_{40}O_7$), D (**4**) ($M^+ + Na$: *m/z* = 679.3130, $C_{36}H_{48}O_{11}Na$) and F (**6**) (M^+: *m/z* = 514.2963, $C_{30}H_{42}O_7$) could be related to those of the sodagnitins A, C, and E, respectively, by replacement of the 12-CH_2 group by an oxo function. 12-Oxomalabaricane derivatives have been isolated before from the marine sponges *Jaspis stellifera*[6] and *Stelletta tenuis*.[7]

The presence of the same side chain in the sodagnitins A, B, E, and F is indicated by the formation of a characteristic intense fragment ion at *m/z* = 167.0740 ($C_9H_{11}O_3$) in the EIMS that results from retro-Diels–Alder cleavage of the 5,6-dihydro(2*H*)pyran ring with concomitant hydrogen transfer.

Triterpenoids with the malabaricane skeleton (Figure 4) are already known from the plants *Ailanthus malabarica*[8] and *Pyrethrum santolinoides*[9] and from two marine sponges.[10,11] The sodagnitins differ from these compounds in their configuration at C-13.

The 5,6-dihydro(2*H*)pyran-2-ol system of the sodagnitins is present in luffariolide D from a marine sponge,[12] furaquinocin G from a *Streptomyces* species[13] and perenniporiol from the wood-rotting fungus *Perenniporia ochroleuca*.[14] The 3(2*H*)furanone moiety is found in the plant metabolites bullatenone,[15] geiparvarin,[16] and wallemia C.[17] The ^{1}H- and ^{13}C-NMR data of the relevant parts of these compounds are in excellent agreement with the corresponding signals of the sodagnitins.

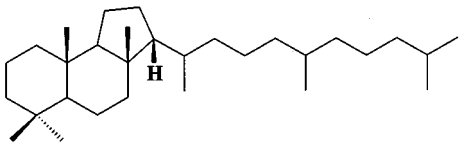
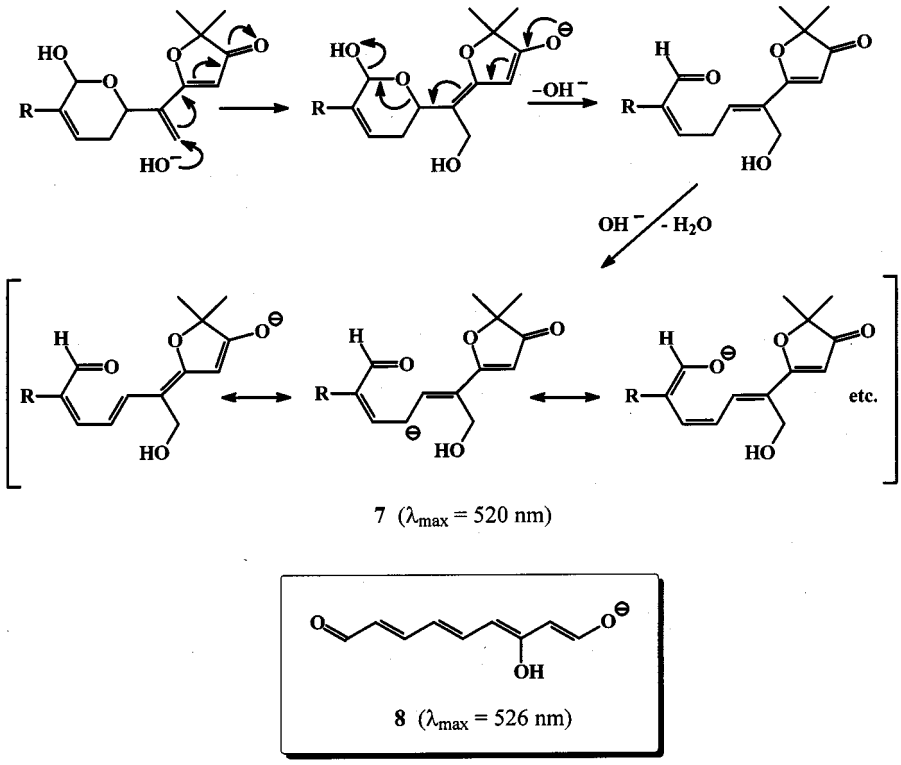


Figure 4. Malabaricane

The sodagnitins are colourless, amorphous powders which can be kept for several months at room temperature. Addition of alkali to their alcoholic solutions immediately causes the development of the characteristic red colour, which persists for several hours. The UV/Vis maximum is thereby shifted from 292 (log ε = 3.78) to 520 nm (4.01). The colour change can be explained by the formation of a highly delocalized oxonol-anion **7** for which we propose the mechanism given in Scheme 1. The reaction is triggered by addition of a hydroxide anion to the *exo*-methylene group of the sodagnitin side chain, followed by a fragmentation with concomitant opening of the tetrahydropyran ring. The resulting 1,9-dioxononatriene moiety then gives rise to the red anion **7**. The λ_{max} values reported for similar oxonol anions[18] are in good agreement with those of alkaline solutions of the sodagnitins. A PPP-π-SCF calculation of the longest wavelength absorption maximum for a geometry optimised oxonol-anion **8** with an oxygen substituent at C-



Scheme 1. Proposed mechanism for the red colour reaction of the sodagnitins with alkali

3 yielded λ_{max}calc. = 526 nm in excellent agreement with the experiment.[19]

Biological Activity

Sodagnitin A and C are active against *Bacillus subtilis*, *Bacillus brevis*, and *Nematospora coryli* at concentrations of 5 μg/disc and higher. Both sodagnitins are cytotoxic against L1210 tumor cells at concentrations higher than 1 and 5 μg/mL, respectively.

Experimental Section

General: Melting points (uncorrected): Reichert hot stage. − Optical rotations: Perkin-Elmer 214. −UV/Vis: Perkin-Elmer Lambda 16. − IR: Perkin-Elmer FT-IR 1000. − NMR: Bruker ARX-300, AMX-600, and Varian VXR-400 S, in $CDCl_3$, with the solvent peak as internal standard. − MS: Finnigan MAT 90 and MAT 95Q. EI spectra were obtained at 70 eV. The (+)- and (−)-FAB spectra were obtained with 5-nitrobenzylic alcohol as matrix. For CI-spectra, isobutane was used as reagent gas. − Solid phase extractions: Chromabond C18 and $(OH)_2$ cartridges (Macherey-Nagel). − TLC: Merck, silica HPTLC plates DIOL F_{254} S, 0.2 mm, solvent system (v/v): toluene/*i*PrOH, 10:1; red spot with methanolic KOH. − Gel chromatography: Sephadex LH-20 (Pharmacia). − Analytical HPLC: Waters 600 E Pump and System Controller with Photodiode Array Detector 990+. System 1: Knauer Vertex column 4 × 250 mm, packing material Nucleosil 100 C18, 5 μm (Macherey-Nagel). Eluent A: H_2O/CH_3CN, 9:1; eluent B: CH_3CN. Linear gradient: 0 min: B 50%, 60 min: B 100%; flow rate 1 mL/min, detection range 200−400 nm. System 2: Knauer Vertex column 4 × 250 mm, packing material Lichrosorb DIOL, 7 μm (Merck). Eluent A: *n*-hexane/*t*BuOMe, 5:1; eluent B: *t*BuOMe/*i*PrOH, 5:1. Linear gradient: 0 min: B 50%, 30 min: B 100%, flow rate 1 mL/min. − Preparative HPLC: Merck Hitachi L 6200 Intelligent Pump and 655A Variable Wavelength UV Monitor. System 1: Knauer Vertex column 16 × 250 mm, packing material Nucleosil 100 C18, 7 μm (Macherey-Nagel). System 2: Knauer Vertex column 16 × 250 mm, Lichrosorb DIOL, 7 μm (Merck). Eluents and gradients as for analytical HPLC. Flow rate 7 mL/min, detection at 290 nm. System 3: Merck column 7 × 250 mm, packing material Lichrogel PS 1 (spheric high performance gel), isocratic elution with *t*BuOMe, flow rate 0.5 mL/min.

Cortinarius sodagnitus was collected in autumn 1992 and the following years in beech forests on limestone near Karlstadt/Main, Bavaria. *Cortinarius arcuatorum* and *Cortinarius* cf. *calochrous* were collected in autumn 1983 and 1988, respectively, in the same region. *C. fulvoincarnatus* was found in autumn 1994 in *Carpinus betulus* forests on limestone in the Wolfsee nature reserve near Uffenheim, Bavaria.

Isolation of the Sodagnitins A−F (1−6) from *C. sodagnitus:* 21 lyophilized fruit-bodies (28 g) were extracted (3×) with EtOH (700 mL). The combined yellow extracts were evaporated and the oily orange residue was distributed between EtOAc/H_2O. The combined organic phases were dried ($MgSO_4$), evaporated and dissolved in *i*PrOH. Chromatography on Sephadex LH-20 with *i*PrOH as eluent afforded several fractions which were monitored by the red-colour reaction with methanolic KOH. The positive fractions were combined and evaporated to dryness. The yellow oily residue was dissolved in *t*BuOMe and an insoluble white residue (fatty acids, steroids) was removed by filtration through a diol cartridge. Preparative HPLC (diol phase, system 2) delivered the single crude sodagnitins. The final purification of the components was achieved by gel permeation chromatography on a styrol gel (system 3) and removal of the solvent in vacuo. The pure sodagnitins A−F (**1**−**6**)

were obtained as slightly yellow, oily films by evaporation of the solvent or as fluffy white powders after lyophilization. The sodagnitins are soluble in organic solvents (e.g. MeOH, EtOAc, $CHCl_3$, and CH_3CN) and are stable at neutral pH. On addition of acids or bases they are rapidly and irreversibly converted into inseparable mixtures. All sodagnitins exhibit the characteristic red KOH-reaction. Yields: 5 mg of **1** (0.018%), 2 mg of **2** (0.007%), 5 mg of **3** (0.018%), 1 mg of **4** (0.003%), 2 mg of **5** (0.007%), 0.5 mg of **6** (0.002%). On TLC using DIOL phase the sodagnitins A-F show decreasing R_f values.

Isolation of the Sodagnitins A (1) and C (3) from *C. fulvoincarnatus*: 40 lyophylized fruit-bodies (70 g) were extracted (5×) with EtOAc (500 mL). The extracts were evaporated to dryness and the residue dissolved in MeOH. Preliminary purification was performed by chromatography on Sephadex LH-20 with MeOH as eluent. The KOH-active fractions were combined and evaporated to dryness. The yellow oily residue was dissolved in MeOH, filtered through a RP 18 cartridge and subjected to preparative HPLC (RP 18 phase, system 1). The pure sodagnitins A and C precipitated after removal of the CH_3CN in vacuo at 40°C. Consecutive freeze-drying of the aqueous suspension delivered the sodagnitins as fluffy white powders. Yields: 32 mg of **1** (0.046%), 201 mg of **3** (0.287%).

Isolation of Sodagnitin C (3) from *C. arcuatorum:* From 3 fresh fruit-bodies (≈ 5 g lyophilized) 18 mg (0.36%) of sodagnitin C were obtained.

Sodagnitin A (1): Amorphous colourless powder. – M.p. 99–102°C (dec.). – $[\alpha]_D^{25} = -35$ (c = 0.41, MeOH). – TLC: R_f = 0.62. – HPLC: t_R = 15.3 min (system 1), 4.5 min (system 2). – UV (MeOH): λ_{max} (lg ε) = 230 nm (3.90), 292 (3.95). – IR (KBr): $\tilde{\nu}$ = 3435 cm^{-1} (br. s), 2932 (m, sh), 1702 (s), 1631 (m), 1557 (m), 1458 (w), 1384 (w), 1255 (w), 1176 (m), 1080 (m), 1023 (m). – ^1H and ^{13}C NMR (see Tables 1 and 2). – EI-MS; *m/z* (%): 498 (16) [M^+], 480 (100), 465 (25), 452 (19), 447 (12), 332 (14), 263 (12), 245 (51), 167 (36). – (+)-FAB-MS; *m/z* (%): 499 (19) [M^+ + H], 481 (7), 308 (8), 307 (31), 289 (16), 257 (8). – (–)-FAB-MS; *m/z* (%): 497 (5) [M^- – H], 459 (9), 307 (11), 306 (55), 305 (48), 304 (11), 199 (22), 168 (34), 166 (11). – CI-MS; *m/z* (%): 499 (2) [M^+ + H], 389 (4), 334 (23), 333 (100), 307 (10), 279 (13), 277 (23), 263 (43), 167 (22). – $C_{30}H_{42}O_6$: calcd. 498.2981; found 498.2989 (HR-EIMS).

Sodagnitin B (2): Amorphous colourless powder. – M.p. 97–100°C (dec.) – TLC: R_f = 0.50. – HPLC: t_R = 6.4 min (system 1), 7.7 min (system 2). – UV (MeOH): λ_{max} ($Abs_{rel.}$) = 230 nm (60), 292 (100). – IR (KBr): $\tilde{\nu}$ = 3436 cm^{-1}(br. s), 2926 (w), 1631 (m, sh), 1384 (w), 1129 (w). – ^1H NMR (600 MHz, $CDCl_3$): δ = 0.91 (s, 3 H), 1.17 (s, 3 H), 1.21 (s, 3 H), 1.27 (s, 3 H), 1.31 (s, 1 H), 1.41 (s, 6 H), 1.8–1.6 (m), 1.92 (ddd, J = 12, 4, 4 Hz, 1 H), 2.4–2.1 (m), 2.88 (s, 1 H), 3.01 (d, J = 6 Hz, 1 H), 3.63 (d, J = 3 Hz, 1 H), 4.59 (m, 1 H), 4.79 (dd, J = 7, 7 Hz, 1 H), 5.26 (d, J = 6 Hz, 1 H), 5.76 (s, 1 H), 5.78 (br. s, 1 H), 6.18 (s, 1 H). – ^{13}C NMR (75 MHz, $CDCl_3$): δ = 16.2 (q), 16.3 (q), 19.7 (t), 21.0 (q), 23.1 (q), 24.7 (q), 27.0 (q), 29.9 (t), 35.7 (t), 36.7 (s), 39.8 (t), 44.3 (s), 47.9 (s), 49.1 (t), 56.5 (d), 57.8 (d), 64.8 (d), 66.0 (d), 69.0 (d), 87.9 (s), 91.3 (d), 100.0 (d), 121.8 (t), 128.7 (d), 130.3 (s), 138.8 (s), 181.1 (s), 207.4 (s), 213.7 (s), 215.3 (s). – EI-MS; *m/z* (%): 512 (7) [M^+], 494 (31), 479 (14), 466 (19), 451 (9), 346 (11), 273 (17), 244 (100), 231 (52), 193 (69), 177 (37), 167 (37). – (+)-FAB-MS; *m/z* (%): 535 (1) [M^+ + Na], 513 (12) [M^+ + H], 495 (16), 307 (23), 289 (12). – (–)-FAB-MS; *m/z* (%): 511 (3) [M^- – H], 345 (5), 306 (41), 305 (42), 199 (22). – CI-MS; *m/z* (%): 513 (2) [M^+ + H], 349 (26), 348 (25), 347 (100), 331 (11), 321 (7), 169 (23), 167 (87) – $C_{30}H_{40}O_7$: calcd. 512.2774; found 512.2823 (HR-EIMS).

Sodagnitin C (3): Amorphous colourless powder. – M.p. 116–119°C (dec.) – $[\alpha]_D^{21} = -15$ (c = 1.00, MeOH). – TLC: R_f = 0.35. – HPLC: t_R = 5.7 min (system 1), 12.4 min (system 3). – UV (MeOH): λ_{max} (lg ε) = 230 nm (3.58), 292 (3.78). – IR (KBr): $\tilde{\nu}$ = 3435 cm^{-1} (br. s), 2975 (s), 2938 (s), 2877 (w), 1730 (s), 1720 (s), 1705 (s), 1631 (w), 1556 (s), 1458 (w), 1386 (m), 1177 (m, sh), 1081 (m), 1018 (w). – ^1H and ^{13}C NMR (see Tables 1 and 2). – (+)-FAB-MS; *m/z* (%): 687 (4) [M^+ – H + 2Na], 666 (23), 665 (57) [M^+ + Na], 643 (3) [M^+ + H], 626 (12), 625 (28), 499 (8), 481 (25). – (–)-FAB-MS; *m/z* (%): 641 (97) [M^- – H], 497 (2), 413 (1), 373 (3), 331 (3), 306 (35), 305 (33). – CI-MS; *m/z* (%): 289 (10), 271 (9), 145 (100). – (+)-APCI-MS; *m/z* (%): 643 (88) [M^+ + H], 625 (100), 513 (39). – $C_{36}H_{50}O_{10}$. – Calcd. for $C_{36}H_{51}O_{10}$: 643.3482; found 643.3491 (HR-APCIMS).

Sodagnitin D (4): Amorphous colourless powder. – M.p. 117–120°C (dec.) – $[\alpha]_D^{23} = +57$ (c = 0.06, MeOH). –TLC: R_f = 0.34. – HPLC: t_R = 4.1 min (system 1), 13.8 min (system 3). – UV (MeOH): λ_{max} (lg ε) = 230 nm (3.70), 292 (3.83). – IR (KBr): $\tilde{\nu}$ = 3435 cm^{-1} (br. s), 2931 (w, sh), 1723 (m, sh), 1631 (m), 1557 (w), 1384 (w), 1178 (w), 1127 (w), 1081 (w), 1029 (w). – ^1H NMR (600 MHz, $CDCl_3$): δ = 0.89 (s, 3 H), 1.15 (s, 3 H), 1.17 (s, 3 H), 1.27 (s, 1 H), 1.29 (s, 3 H), 1.39 (s, 6 H), 1.48 (s, 3 H), 1.57 (m), 1.69 (m, 2 H), 1.91 (d, J = 12 Hz, 1 H), 2.10 (dd, J = 12, 6 Hz, 1 H), 2.20 (m), 2.33 (m, 2 H), 2.66 (m, 2 H), 2.74 (m, 2 H), 2.87 (s, 1 H), 3.05 (m, 1 H), 4.76 (dd, J = 7, 7 Hz, 1 H), 5.24 (s, 1 H), 5.71 (dd, J = *1*3, 6 Hz, 1 H), 5.74 (br. s, 1 H), 5.76 (s, 1 H), 6.17 (s, 1 H). – (+)-FAB-MS; *m/z* (%): 679 (12) [M^+ + Na], 657 (2) [M^+ + H], 639 (19), 495(2). – (–)-FAB-MS; *m/z* (%): 655 (38) [M^- – H], 511 (0.3). – CI-MS; *m/z* (%): 349 (0.4), 347 (0.6), 321 (0.8), 289 (0.5), 285 (0.6), 279 (0.9), 271 (0.5), 145 (100), 127 (23), 101 (16). – $C_{36}H_{48}O_{11}$. –- Calcd. for $C_{36}H_{48}O_{11}Na$ [M^+ + Na]: 679.3094; found: 679.3130 (HR-ESIMS).

Sodagnitin E (5): Amorphous colourless powder. – M.p. 105–108°C (dec.). – TLC: R_f = 0.25. – HPLC: t_R = 9.1 min (system 1), 8.5 min (system 2). – UV (MeOH): λ_{max} (lg ε) = 230 nm (3.37), 292 (3.49). – IR (KBr): $\tilde{\nu}$ = 3435 cm^{-1} (br. s), 2929 (w, sh), 1686 (m), 1631 (m, sh), 1558 (w), 1457 (w), 1384 (w), 1177 (w), 1077 (w), 1044 (w). – ^1H and ^{13}C NMR (see Tables 1 and 2). – EI-MS; *m/z* (%): 500 (15), 482 (21), 277 (13), 263 (26), 245 (19), 233 (27), 223 (18), 205 (36), 193 (22), 177 (37), 167 (42); (+)-FAB-MS; *m/z* (%): 523 (6) [M^+ + Na], 503 (5), 502 (19), 501 (56) [M^+ + H], 500 (5) [M^+], 499 (7), 484 (15), 483 (41), 395 (19), 377 (19), 307 (19), 289 (17). – (–)-FAB-MS; *m/z* (%): 499 (7) [M^- – H], 333 (5), 306 (25), 305 (26), 199 (15). – CI-MS (isobutane); *m/z* (%): 501 (0.3) [M^+ + H], 335 (17), 317 (26), 277 (13), 169 (21), 167 (45), 145 (100). – $C_{30}H_{44}O_6$: calcd. 500.3138; found 500.3138 (HR-EIMS).

Sodagnitin F (6): Amorphous colourless powder. – M.p. 115–118°C (dec.). – TLC: R_f = 0.21. – HPLC: t_R = 4.9 min (system 1), 13.6 min (system 2). – UV (MeOH): λ_{max} (lg ε) = 230 nm (3.46), 292 (3.56). – IR (KBr): $\tilde{\nu}$ = 3435 cm^{-1} (br. s), 2925 (w), 1720 (m), 1681 (m), 1631 (m, sh), 1558 (w), 1384 (w), 1178 (w), 1130 (w), 1045 (w). – ^1H NMR (600 MHz, $CDCl_3$): δ = 0.83 (s, 3 H), 0.86 (s, 3 H), 0.98 (s, 3 H), 1.04 (s, 3 H), 1.39 (s, 6 H), 1.67 (dd, J = 14, 7 Hz, 1 H), 1.71 (m, 1 H), 1.85 (m, 2 H), 2.11 (m), 2.21 (dd, J = 18, 7 Hz, 1 H), 2.33 (m, 2 H), 2.86 (s, 1 H), 2.99 (d, J = 6 Hz, 1 H), 3.03 (dd, J = 9, 1 Hz, 1 H), 3.74 (m, 1 H), 4.76 (dd, J = 7, 7 Hz, 1 H), 5.23 (d, J = 6 Hz, 1 H), 5.73 (d, J = 8 Hz, 1 H), 5.75 (s, 1 H), 5.77 (s, 1 H), 6.16 (s, 1 H). – EI-MS; *m/z* (%): 514 (6) [M^+], 496 (30), 481 (11), 467 (5), 348 (19), 291 (10), 273 (17), 249 (21), 244 (67), 231 (41), 205 (91), 193 (100), 177 (30), 167 (45). – (+)-FAB-MS; *m/z* (%): 537 (1) [M^+ + Na], 515

(5) [M^+ + H], 498 (4), 497 (11), 307 (28), 290 (10), 289 (21), 273 (13). – (–)-FAB-MS; *m/z* (%): 513 (3) [M^- – H], 459 (12), 458 (7), 347 (3), 307 (11), 306 (60), 305 (48), 304 (11). – $C_{30}H_{42}O_7$: calcd. 514.2931; found 514.2963 (HR-EIMS).

Acknowledgments

We thank the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie for support of this research. We are grateful to Prof. T. Anke, University of Kaiserslautern, for the biological tests and to Drs. Norbert Arnold and Bernhard Oertel for their kind help in collecting the toadstools. We are also indebted to Dr. Bert Steffan for NMR experiments. The linguistic help of Dr. Melvyn Gill, University of Melbourne, in preparing the manuscript is gratefully acknowledged.

[1] M. Moser, *Die Gattung Phlegmacium (Schleimköpfe)* in: *Die Pilze Mitteleuropas, Vol. 4*, Verlag Julius Klinkhardt, Bad Heilbrunn, **1960**.
[2] B. Oertel, D. Laber, *Z. Mykol.* **1996**, *52*, 139–154.
[3] A. Dossena, A. Lunghi, L. Garlaschelli, G. Vidari, *Tetrahedron: Asymmetry* **1996**, *7*, 1911–1914.
[4] [4a] W. F. Tinto, L. M. D. John, W. F. Reynolds, S. McLean, *J. Nat. Prod.* **1992**, *55*, 807–809. – [4b] V. V. Velde, D. Lavie, *Tetrahedron* **1983**, *39*, 317–321.
[5] [5a] A. L. Wilkins, J. Bremer, J. Ralph, P. T. Holland, K. J. Ronaldson, P. M. Jager, P. W. Bird, *Aust. J. Chem.* **1989**, *42*, 243–257. – [5b] M. De Bernardi, G. Fronza, M. P. Gianotti, G. Mellerio, G. Vidari, P. Vita–Finzi, *Tetrahedron Lett.* **1983**, *24*, 1635–1638.
[6] [6a] B. N. Ravi, R. J. Wells, K. D. Croft, *J. Org. Chem.* **1981**, *46*, 1998–2001. – [6b] B. N. Ravi, R. J. Wells, *Aust. J. Chem.* **1982**,*35*, 39–50. – [6c] M. Tsuda, M. Ishibashi, K. Agemi, T. Sasaki, J. Kobayashi, *Tetrahedron* **1991**, *47*, 2181–2194.
[7] [7a] T. McCabe, J. Clardy, L. Minale, C. Pizza, F. Zollo, R. Riccio, *Tetrahedron Lett.* **1982**, *23*, 3307–3310. – [7b] J. Y. Su, Y. H. Meng, L. M. Zeng, X. Fu, F. J. Schmitz, *J. Nat. Prod.* **1994**, *57*, 1450–1451.
[8] [8a] A. Chawla, S. Dev, *Tetrahedron Lett.* **1967**, *48*, 4837–4843. – [8b] R. R. Sobti, S. Dev, *Tetrahedron Lett.* **1968**, *18*, 2215–2217.
[9] J. Jakupovic, F. Eid, F. Bohlmann, S. El-Dahmy, *Phytochemistry* **1987**, *26*, 1536–1538.
[10] H. Ageta, K. Masuda, M. Inoue, T. Ishida, *Tetrahedron Lett.* **1982**, *23*, 4349–4352.
[11] K. Iguchi, Y. Shimada, Y. Yamada, *J. Org. Chem.* **1992**, *57*, 522–524.
[12] [12a] E. D. de Silva, P. J. Scheuer, *Tetrahedron Lett.* **1981**, *22*, 3147–3150. – [12b] M. Tsuda, H. Shigemori, M. Ishibashi, T. Sasaki, J. Kobayashi, *J. Org. Chem.* **1992**, *57*, 3503–3507.
[13] M. Ishibashi, S. Funayama, Y. Anraku, K. Komiyama, S. Omura, *J. Antibiot.* **1991**, *44*, 390–395.
[14] M. Hirotani, C. Ino, T. Furuya, M. Shiro, *Phytochemistry* **1984**, *23*, 1129–1134.
[15] [15a] C. W. Brandt, M. I. Taylor, B. R. Thomas, *J. Chem. Soc.* **1954**, 3245–3247. – [15b] A. B. Smith, P. A. Levenberg, P. J. Jerris, R. M. Scarborough, P. M. Wovkulich, *J. Am. Chem. Soc.* **1981**, *103*, 1501–1513.
[16] [16a] F. N. Lahey, J. K. McLeod, *Aust. J. Chem.* **1967**, *20*, 1943–1955. – [16b] T. Sakai, K. Kohda, S. Tsuboi, M. Utaka, A. Takeda, *Bull. Chem. Soc. Jpn.* **1987**, *60*, 2911–2915.
[17] Y. Badar, W. J. S. Lockley, T. P. Toube, B. C. L. Weedon, L. R. G. Valadon, *J. Chem. Soc., Perkin Trans. 1* **1973,** 1416–1424.
[18] M. Klessinger, *Chem. Unserer Zeit* **1978**, *1*, 1–11.
[19] Structure optimisation of the oxonol anion geometry was performed by a MNDO calculation using the program AMSOL with AM 1 parameters. The determination of the excited states (only singlet states) was carried out by a PPP (Pariser–Parr–Pople) calculation using the program WPSIN, based on a π Self Consistant Field method. We are grateful to Dr. *H.-U. Wagner*, LMU München, for carrying out the calculations and for helpful discussions.

Received August 6, 1998
[O98373]