

Novel Diterpenoids from *Salvia dugesii*

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Two novel rearranged clerodane diterpenoids, dugesin A (**1**) and dugesin B (**2**), were isolated from the aerial parts of *Salvia dugesii*, together with five known clerodane diterpenoids: isosalvipuberulin (**3**), salvipuberulin (**4**), tilifodiolide (**5**), salvifolin (**6**), and salvifaricin (**7**). Their structures were elucidated on the basis of different spectroscopic techniques. The isolation and identification of these compounds are significant from both biosynthesis and chemotaxonomy points of view.

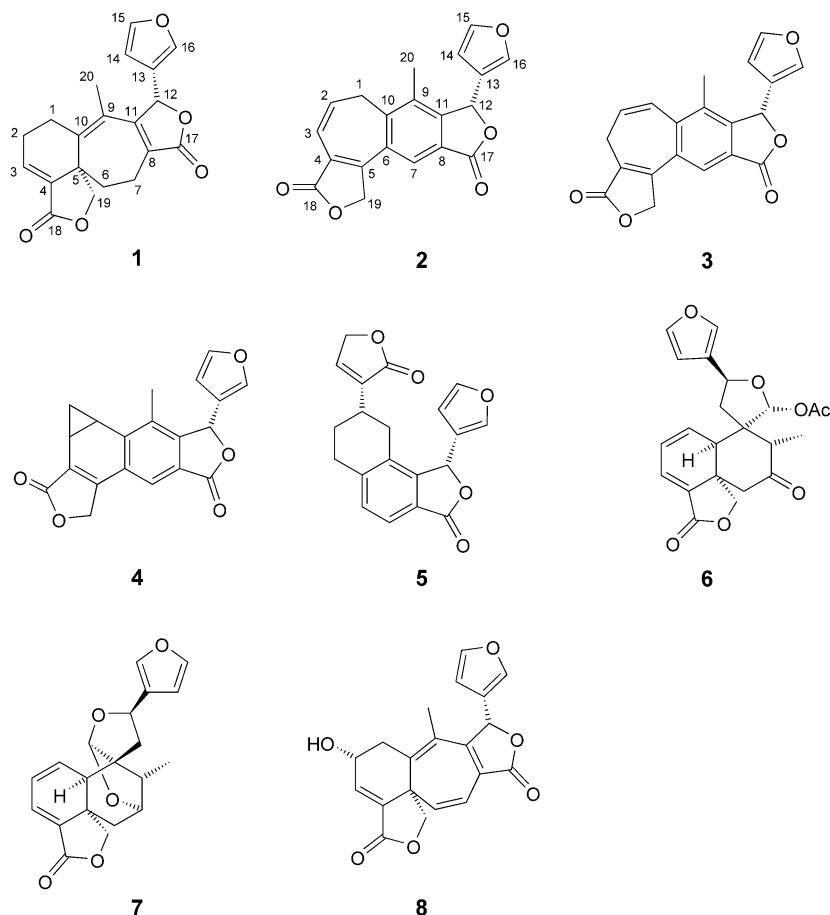
Introduction. – *Salvia*, which includes 700–1050 species worldwide, is the largest genus in the Labiatae family [1]. A variety of bi- and tricyclic diterpenoids have been isolated from the species of this genus. Systematic chemotaxonomic study of the species of the *Salvia* subgenus *Calosphace* has revealed an interesting relationship between the diterpenoid content of the species under study and the section to which it belongs [2]. A member of this subgenus, *S. dugesii*, has not been investigated chemically so far. Therefore, we decided to analyze its chemical components and the relationship between *S. dugesii* and the other species of the subgenus *Calosphace*.

Results and Discussion. – Extraction of the aerial parts of *S. dugesii* afforded, after extensive chromatographic purification, two new diterpenoids of clerodane origin, dugesin A (**1**) and dugesin B (**2**), as well as five known diterpenoids: isosalvipuberulin (**3**) [2][3], salvipuberulin (**4**) [3], tilifodiolide (**5**) [2], salvifolin (**6**) [2], and salvifaricin (**7**) [4][5]. Dugesin A (**1**) is, to our best knowledge, the fourth diterpenoid isolated from a natural source with this type of a salvigenane skeleton [6], and dugesin B is the second natural product of clerodanic origin with a benzocycloheptatriene structure [3].

The structures of the five known compounds **3–7** were established by comparing their MS and NMR spectral data with those reported in the literature. Compounds **2–5** and **7** have been screened for their antitumor properties (CDC25; Cell Division Circle 25), but none of them showed remarkable activity.

It is noteworthy that tilifodiolide (**5**; >0.2%), the major constituent of *S. dugesii*, was the only tetraline-type diterpenoid of clerodanic origin; it has been previously isolated from *S. tiliaefolia* [2]. Salvipuberulin (**4**), previously isolated from *S. puberula*, is also the only natural product of clerodane diterpenoids with a benzonorcaradiene structure [3].

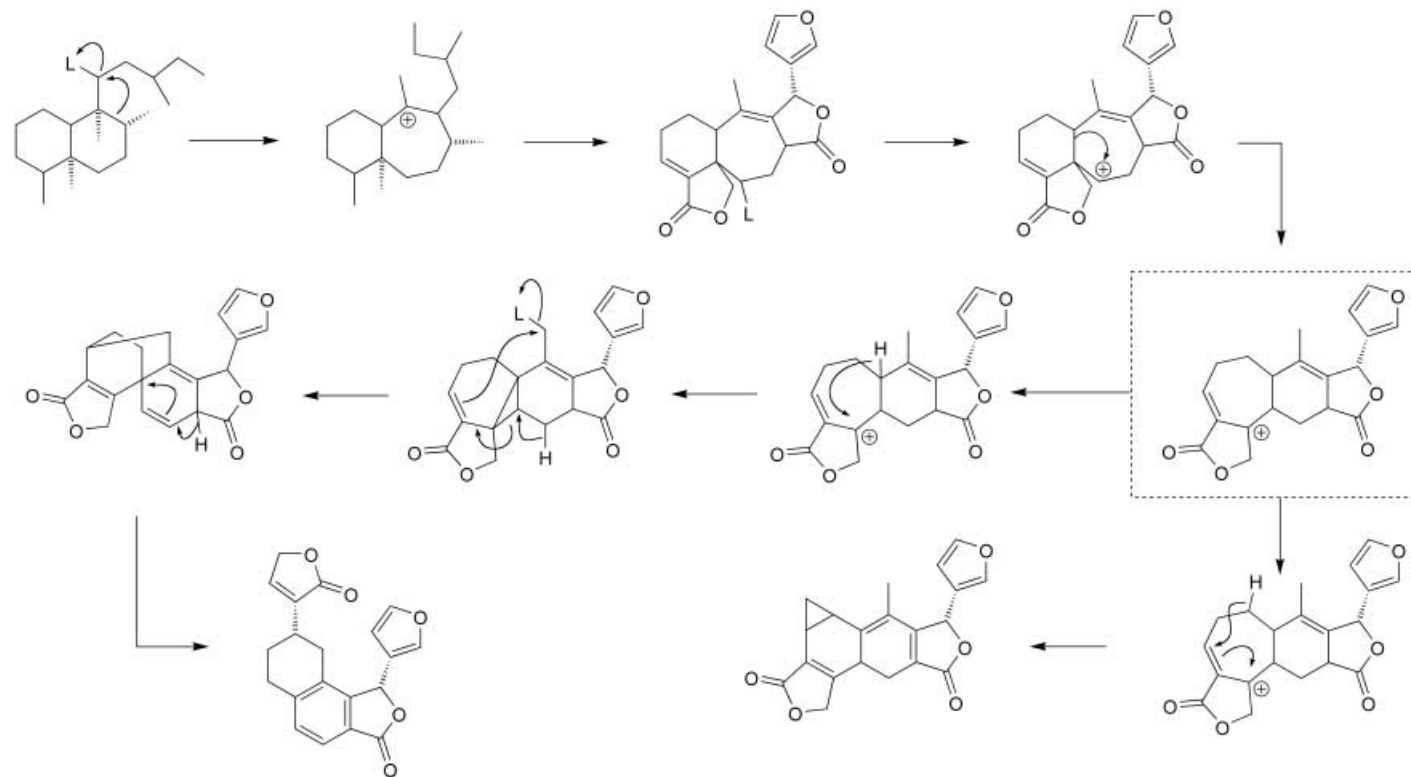
It is interesting that all the above diterpenoids were isolated from the same plant. The diterpenoids from *S. dugesii* are clerodanic diterpenoids, or diterpenoids with rearranged skeletons biogenetically related to a normal clerodane precursor. Their



biogenesis has been proposed repeatedly (*Scheme*) [2][3][7], but, until now, they have never been isolated from the same plant. The isolation of these different types of diterpenoids may suggest that *S. dugesii* is a special case in chemotaxonomic terms [2][8]. Its diterpenoids are very similar to those of *S. tiliaefolia* and *S. puberula*, both belonging to the subgenus *Calosphaea*.

Compound **1** was isolated as a white amorphous powder. Its molecular formula, $C_{20}H_{18}O_5$, was determined by HR-EI-MS (m/z 338.123 (M^+ ; calc. 338.115)) and NMR spectral data. The IR spectrum indicated the presence of a furan ring (1503 and 874 cm^{-1}) and an α,β -unsaturated γ -lactone (1755 and 1686 cm^{-1}). The $^1\text{H-NMR}$ spectral data are given in *Table 1*. There were characteristic signals due to a β -substituted furan ring at δ_{H} 6.52 (br. s, H–C(14)), 7.60 (br. s, H–C(15)), and 7.80 (br. s, H–C(16)) [3]. Corresponding signals in the $^{13}\text{C-NMR}$ spectrum (*Table 1*) were observed at δ_{C} 121.6 (s, C(13)), 109.0 (d, C(14)), 144.5 (d, C(15)), and 142.2 (d, C(16)). HMQC Correlations between the signals at δ_{H} 6.23 (br. s, 1 H) and δ_{C} 75.5 (d) indicated an O-bearing methine group (H–C(12)). Two lactone C=O groups were

Scheme. *Proposed Biogenetic Pathway to Rearranged Clerodane Diterpenoids* [2][3][7]. Leaving groups (L) are indicated.



found at δ_C 167.3 and 168.4. In the mass spectrum of **1**, the presence of an intense fragment signal at m/z 95 (78%) supported a γ -lactone and a furan moiety bound to C(12) [2][3]. These spectral features indicated that compound **1** was a rearranged clerodane diterpenoid [2][3][6][7]. By extensive analysis of the IR, MS, and NMR spectral data and comparison with those of diterpenoids isolated from *Calosphaace* [2][3][6][7], **1** was assumed to be a rearranged clerodane diterpenoid closely related to salviandulin E (**8**), which has been previously isolated from *S. Leucantha* [6]. The ^{13}C -NMR spectral data of **1** were very similar to those of **8**, except for the presence of three additional methylene signals at δ_C 22.8 (*t*), 35.3 (*t*), and 22.6 (*t*), and the absence of one saturated, O-bearing methine group (C(2) of **8**) and two unsaturated methine groups (C(6) and C(7) of **8**).

Table 1. ^1H - and ^{13}C -NMR Data (CDCl_3 ; 400 or 50 MHz, resp.) of *Dugesin A* (**1**). δ in ppm, *J* in Hz.

	δ_C	δ_H	COSY ($^1\text{H}, ^1\text{H}$)	HMBC ($^1\text{H}, ^{13}\text{C}$)
H _a -C(1)	29.8 (<i>t</i>)	2.92 (<i>m</i>)	H _b -C(1), H _a -C(2), H _b -C(2)	C(2), C(3), C(5)
H _b -C(1)		2.05 (<i>m</i>)	H _a -C(1), H _a -C(2), H _b -C(2), H-C(20)	C(2), C(3), C(5), C(9), C(10)
H _a -C(2)	22.8 (<i>t</i>)	2.48 (<i>m</i>)	H _a -C(1), H _b -C(1), H _b -C(2), H-C(3)	C(1), C(3), C(4), C(10)
H _b -C(2)		2.30 (<i>m</i>)	H _a -C(1), H _b -C(1), H _a -C(2), H-C(3)	C(1), C(10)
H-C(3)	136.3 (<i>d</i>)	7.07 (<i>dd</i> , <i>J</i> = 2.4, 6.2)	H _a -C(2), H _b -C(2)	C(1), C(2), C(5), C(18)
C(4)	135.5 (<i>s</i>)			
C(5)	46.9 (<i>s</i>)			
H _a -C(6)	35.3 (<i>t</i>)	2.04 (<i>m</i>)	H _b -C(6), H _a -C(7), H _b -C(7)	C(5), C(7), C(8), C(10)
H _b -C(6)		2.30 (<i>m</i>)	H _a -C(6), H _a -C(7), H _b -C(7)	C(5), C(7), C(10), C(19)
H _a -C(7)	22.6 (<i>t</i>)	2.70 (<i>m</i>)	H _a -C(6), H _b -C(6), H _b -C(7)	C(5), C(6), C(8), C(11)
H _b -C(7)		2.30 (<i>m</i>)	H _a -C(6), H _b -C(6), H _a -C(7)	C(5)
C(8)	127.5 (<i>s</i>)			
C(9)	124.5 (<i>s</i>)			
C(10)	147.3 (<i>s</i>)			
C(11)	159.7 (<i>s</i>)			
H-C(12)	75.5 (<i>d</i>)	6.23 (<i>br. s</i>)		C(8), C(11), C(13), C(14)
C(13)	121.6 (<i>s</i>)			
H-C(14)	109.0 (<i>d</i>)	6.52 (<i>br. s</i>)	H-C(15), H-C(16)	C(13), C(15), C(16)
H-C(15)	144.5 (<i>d</i>)	7.60 (<i>br. s</i>)	H-C(14), H-C(16)	C(13), C(14), C(16)
H-C(16)	142.2 (<i>d</i>)	7.80 (<i>br. s</i>)	H-C(14), H-C(15)	C(13), C(14), C(15)
C(17)	167.3 (<i>s</i>)			
C(18)	168.4 (<i>s</i>)			
H _a -C(19)	71.1 (<i>t</i>)	4.82 (<i>d</i> , <i>J</i> = 7.2)	H _b -C(19)	C(4), C(5), C(6), C(18)
H _b -C(19)		3.70 (<i>d</i> , <i>J</i> = 7.2)	H _a -C(19)	C(4), C(5),
H-C(20)	16.5 (<i>q</i>)	1.89 (<i>s</i> , 3 H)		C(9), C(10), C(11)

Comparison of the ^1H -NMR data of compounds **1** and **8** further confirmed the proposed structure of **1** (absence of two olefinic H-atoms, presence of an additional signal for an O-bearing methine group). In the HMBC spectrum, an olefinic H-atom at δ_H 7.07 (*dd*, *J* = 2.4, 6.2, 1 H) coupled with C(18) at δ_C 168.4 (*s*), and was ascribed to

H–C(3). The ^1H , ^1H -COSY correlations between H–C(3) and the signals at δ_{H} 2.92 (*m*, 1 H), 2.05 (*m*, 1 H), 2.48 (*m*, 1 H), and 2.30 (*m*, 1 H) suggested that these four H-atoms could be assigned to H_a –C(1), H_b –C(1), H_a –C(2), and H_b –C(2), respectively. Therefore, obviously, C(1) (δ_{C} 29.8 (*t*)) and C(2) (22.8 (*t*)) had to be two methylene groups in **1**. Moreover, HMBC correlations between H–C(19), at δ_{H} 4.82 and 3.70 (*2dd*, $J = 7.2$, 2×1 H)), and a CH_2 C-atom (δ_{C} 35.3 (*t*)) were found. In the same experiment, this methylene group at δ_{H} 2.04 and 2.30 (*2m*, 2×1 H) was coupled with C(5) (δ_{C} 46.9 (*s*)), C(8) (127.5 (*s*)), C(10) (147.3 (*s*)), and another CH_2 group at δ_{C} 22.6 (*t*). These HMBC correlations suggested that these two CH_2 groups could be ascribed to C(6) and C(7), respectively. This assignment was confirmed by correlation signals between H–C(6) and H–C(7) in the corresponding COSY spectrum. Hence, based on the evidence described, dugesin A was represented by structure **1**.

Dugesin B (**2**), obtained as colorless crystals from acetone, had a molecular formula of $\text{C}_{20}\text{H}_{14}\text{O}_5$, as determined by HR-EI-MS (m/z 334.095; calc.: 334.084) and NMR spectral data. Its IR spectrum showed bands assigned to an α,β -unsaturated γ -lactone (1754 and 1655 cm^{-1}), a β -substituted furan (1508 and 874 cm^{-1}), and an aromatic system (1605 and 1625 cm^{-1}). By extensive analysis of the IR, MS, and NMR spectral data (Table 2), and by comparison with the data of the diterpenoids isolated from the same subgenus [2][3], **2** was assumed to have the same rearranged clerodane-type skeleton as the isolated isosalvipuberulin (**3**), which has also been isolated from *salvia puberula* [3].

Table 2. ^1H - and ^{13}C -NMR Data (CDCl_3 ; 400 or 50 MHz, resp.) of Dugesin B (**2**). δ in ppm, J in Hz.

	δ_{C}	δ_{H}	COSY (^1H , ^1H)	HMBC (^1H , ^{13}C)
H_a –C(1)	29.7 (<i>t</i>)	3.17 (<i>dd</i> , $J = 7.0$, 13.6)	H_b –C(1), H–C(2)	C(2), C(3), C(6), C(9), C(10)
H_b –C(1)		3.05 (<i>dd</i> , $J = 7.0$, 13.6)	H_a –C(1), H–C(2)	C(2), C(3), C(6), C(9), C(10)
H–C(2)	128.5 (<i>d</i>)	5.98 (<i>td</i> , $J = 7.0$, 9.6)	H_a –C(1), H_b –C(1), H–C(3)	C(1), C(3), C(4), C(10)
H–C(3)	119.7 (<i>d</i>)	6.52 (<i>d</i> , $J = 9.6$)	H–C(2)	C(2), C(4), C(5), C(18)
C(4)	126.5 (<i>s</i>)			
C(5)	155.4 (<i>s</i>)			
C(6)	132.2 (<i>s</i>)			
H–C(7)	124.1 (<i>d</i>)	7.78 (<i>br. s</i>)		C(5), C(6), C(11), C(17)
C(8)	121.9 (<i>s</i>)			
C(9)	131.0 (<i>s</i>)			
C(10)	141.7 (<i>s</i>)			
C(11)	150.4 (<i>s</i>)			
H–C(12)	75.0 (<i>d</i>)	6.43 (<i>br. s</i>)		C(8), C(9), C(11), C(13), C(14), C(16), C(17)
C(13)	120.6 (<i>s</i>)			
H–C(14)	108.6 (<i>d</i>)	6.04 (<i>br. s</i>)	H–C(15)	C(12), C(13), C(15)
H–C(15)	144.3 (<i>d</i>)	7.35 (<i>br. s</i>)	H–C(14), H–C(16)	C(13), C(14), C(16)
H–C(16)	142.2 (<i>d</i>)	7.51 (<i>br. s</i>)	H–C(15)	C(13), C(14), C(15)
C(17)	169.3 (<i>s</i>)			
C(18)	172.5 (<i>s</i>)			
H_a –C(19)	69.8 (<i>t</i>)	5.39 (<i>d</i> , $J = 17.5$)	H_a –C(19)	C(4), C(5), C(18)
H_b –C(19)		5.34 (<i>d</i> , $J = 17.5$)	H_b –C(19)	C(4), C(5), C(18)
H–C(20)	15.5 (<i>q</i>)	2.24 (<i>br. s</i> , 3 H)		C(9), C(10), C(11)

The MS, IR, and NMR spectra of compounds **2** and **3** were strikingly similar. In the ^{13}C -NMR spectra, the CH_2 signal at δ_{C} 21.9 (*t*) of **3** was shifted to 29.7 ppm in **2**, suggesting that the $\text{CH}_2(3)$ group in **3** was ‘flipped’, now corresponding to $\text{CH}_2(1)$ in **2** (double-bond shift). The observed difference in the chemical shift of the $\text{CH}_2(1)$ group can be rationalized by the disappearance of the shielding effect from the C(18) C=O group present in **3**.

Based on HMBC correlations between the signals at δ_{H} 3.17 and 3.05 (*ddd*, $J = 7.0$, 13.6, CH_2) and C(6) (δ_{C} 132.2 (*s*)), C(9) (131.0 (*s*)), and C(10) (141.7 (*s*)), these resonances were unambiguously ascribed to $\text{CH}_2(1)$ at δ_{C} 29.7 (*t*) of **2**. Moreover, an HMBC correlation between H–C(3) at δ_{H} 6.52 (*d*, $J = 9.6$) and C(18) at δ_{C} 172.5 (*s*) was observed, indicating that the double bond was between C(2) and C(3). The rest of the spectrum of **2** was identical to that of isosalvipuberulin (**3**) [3]. From these results, we concluded that dugesin B is represented by structure **2**.

Compound **3** was identified as isosalvipuberulin by means of comparison of the corresponding MS and NMR spectra with literature data [3]. However, we found that the literature assignment of C(4) and C(11) was inaccurate [3]. The HMBC correlation observed between H–C(3) (δ_{H} 2.97 and 2.90 (*ddd*, $J = 6.6$, 15.9)) and the signal at δ_{C} 127.8 suggested that the latter had to be assigned to C(4). The signal at δ_{C} 147.2 (*s*) was ascribed to C(11) due to a HMBC correlation with H–C(12) (δ_{H} 6.46 (*br. s*, 1 H)). Our assignment of C(4) and C(11), respectively, can be rationalized by conjugation effects typical for α - and β -olefinic C-atoms of α,β -unsaturated carbonyl compounds.

Experimental Part

General. Melting points (m.p.) were determined with a *Kofler* micro-melting-point apparatus. Optical rotations were measured on a *Horiba Sepa-300* polarimeter. UV Spectra were recorded on a *Shimadzu UV-210A* spectrometer; λ_{max} (log ϵ) in nm. IR Spectra were recorded on a *Perkin-Elmer 577* spectrophotometer with KBr discs; in cm^{-1} . 1D- and 2D-NMR Spectra were recorded on a *Bruker AM-400* spectrometer, with SiMe_4 as internal standard; chemical shifts δ in ppm, J in Hz. Mass spectra (70 eV) were obtained on an *Autospec-3000* spectrometer; in m/z (rel. %).

Plant Material. The dried aerial parts of *Salvia dugesii* were collected in Kunming, P.R. China, and identified by Prof. *Xi-Wen Li* of the Kunming institute of Botany, Chinese Academy of Sciences, Kunming, China, where a voucher specimen (No. 200097) was deposited.

Extraction and Isolation. The dried and powdered aerial parts of *S. dugesii* (7.2 kg) were extracted with acetone (3×168 h) at r.t. The solvent was removed *in vacuo*, and the gummy remainder was subjected to column chromatography (CC) (*D-101* porous resin; MeOH/ H_2O 1:1 and 9:1). The residue of the elution with MeOH/ H_2O 9:1 was partitioned between H_2O and AcOEt, and the org. phase was subjected to CC (SiO_2 ; petroleum ether/acetone of increasing polarity). The product fractions were combined and subjected again to CC (SiO_2 ; CHCl_3 /acetone) to afford compounds **1** (3 mg), **2** (78 mg), **3** (645 mg), **4** (18 mg), **5** (13 mg), **6** (55 mg), and **7** (5 mg).

(8*R*,12*aS*)-8-(Furan-3-yl)-5,6,11,12-tetrahydro-7-methyl-3H-furo[3',4':4,5]cyclohepta[1,2-d][2]benzofuran-3,10(8H)-dione (*Dugesin A*; **1**). White amorphous powder. $[\alpha]_{\text{D}}^{25} = +17.24$ ($c = 0.145$; MeOH). UV (MeOH): 286.4 (0.44), 203.8 (1.05). IR (KBr): 2925, 1755, 1687, 1504, 1452, 1384, 1272, 1200, 1174, 1130, 1084, 1054. ^1H - and ^{13}C -NMR: see *Table 1*. EI-MS: 338 (51, M^+), 306 (9), 292 (29), 277 (21), 240 (100), 212 (22), 205 (20), 189 (32), 183 (55), 165 (45), 152 (58), 141 (34), 128 (45), 115 (50), 95 (78). HR-EI-MS: 338.123 ($\text{C}_{20}\text{H}_{18}\text{O}_5^+$; calc. 338.115).

(8*R*)-8-(Furan-3-yl)-6,8-dihydro-7-methyl-3H-furo[3',4':3,4]cyclohepta[1,2-f][2]benzofuran-3,10(1H)-dione (*Dugesin B*; **2**). Colorless crystals (acetone). M.p. 220–223° (acetone). $[\alpha]_{\text{D}}^{25} = -123.08^\circ$ (CHCl_3 ; $c = 0.325$). UV (CHCl_3): 300.6 (1.48), 246.2 (3.14). IR (KBr): 2860, 2366, 1754, 1625, 1605, 1507, 1351, 1298, 1263, 1146, 1114, 1083, 1025. ^1H - and ^{13}C -NMR: see *Table 2*. EI-MS: 344 (100, M^+), 319 (8), 305 (64), 239 (95), 189 (35), 165 (23), 152 (40), 95 (56). HR-EI-MS: 344.095 ($\text{C}_{20}\text{H}_{14}\text{O}_5^+$; calc. 344.084).

(8R)-8-(Furan-3-yl)-4,8-dihydro-7-methyl-3H-furo[3',4':3,4]cyclohepta[1,2-f][2]benzofuran-3,10(1H)-dione (isosalvipuberulin; **3**). Colorless crystals (acetone). ¹³C-NMR (CDCl₃): 126.9 (d, C(1)); 132.8 (d, C(2)); 21.9 (t, C(3)); 127.8 (s, C(4)); 153.6 (s, C(5)); 133.4 (s, C(6)); 121.3 (d, C(7)); 124.5 (s, C(8)); 131.5 (s, C(9)); 141.8 (s, C(10)); 147.2 (s, C(11)); 75.1 (d, C(12)); 120.7 (s, C(13)); 108.7 (d, C(14)); 144.3 (d, C(15)); 142.2 (d, C(16)); 169.3 (s, C(17)); 172.4 (s, C(18)); 69.4 (t, C(19)); 15.9 (q, C(20)).

REFERENCES

- [1] C. Y. Wu, H. W. Li, *Acta Bot. Yunnan.* **1982**, *4*, 97.
- [2] L. Rodriguez-Hahn, R. O'Reilly, B. Esquivel, E. Maldonado, A. Ortega, J. Cardenas, R. A. Toscano, *J. Org. Chem.* **1990**, *55*, 3522.
- [3] L. Rodriguez-Hahn, B. Esquivel, A.-A. Sanchez, J. Cardenas, O. G. Tovar, M. Soriano-Garcia, A. Toscano, *J. Org. Chem.* **1988**, *53*, 3933.
- [4] G. Savona, D. Raffa, M. Bruno, B. Rodriguez, *Phytochemistry* **1983**, *22*, 784.
- [5] B. Rodriguez, C. Pascual, G. Savona, *Phytochemistry* **1984**, *23*, 1193.
- [6] B. Esquivel, J. Cardenas, A. Toscano, M. Soriano-Garcia, L. Rodriguez-Hahn, *Tetrahedron* **1994**, *50*, 11593.
- [7] B. Esquivel, R. M. Domingues, S. Hernandez-Ortega, R. A. Toscano, L. Rodriguez-Hahn, *Tetrahedron* **1985**, *41*, 3213.
- [8] M. C. Fernandez, B. Esquivel, J. Cardenas, A. A. Sanchez, R. A. Toscano, L. Rodriguez-Hahn, *Tetrahedron* **1991**, *47*, 7199.

Received November 3, 2003