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 biosynthetis and chemotaxonomy points of view.

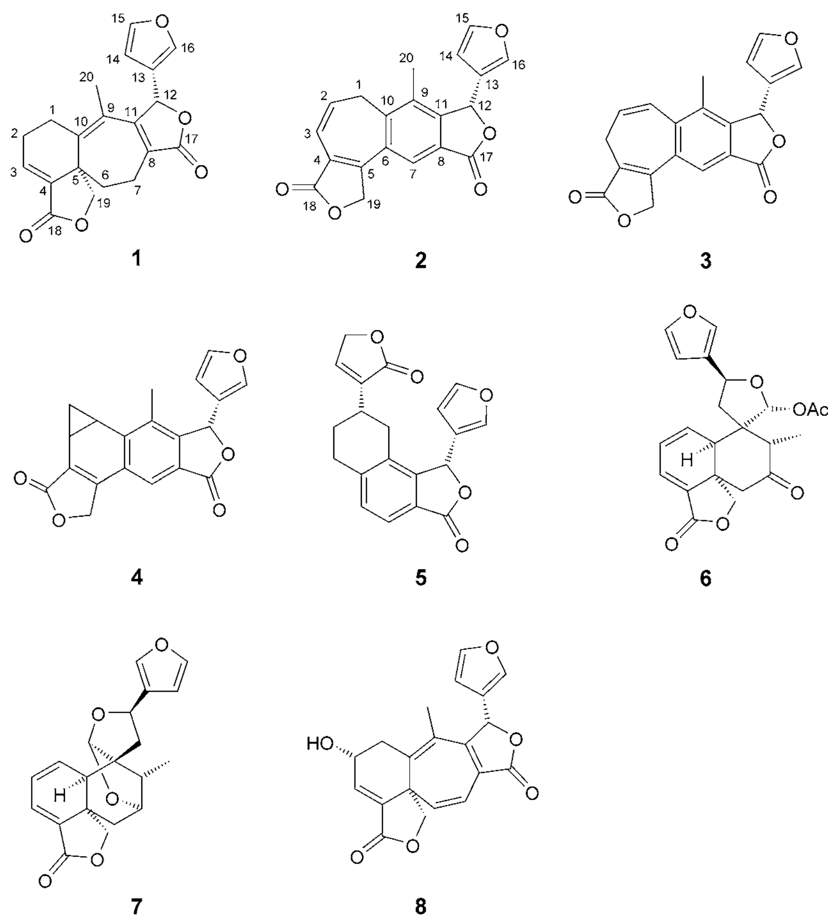
Introduction. – *Salvia*, which includes 700–1050 species worldwide, is the largest genus in the Labiatae family [1]. A variety of bi- and tricyclic diterpenoids have been isolated from the species of this genus. Systematic chemotaxonomic study of the species of the *Salvia* subgenus *Calosphace* has 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].

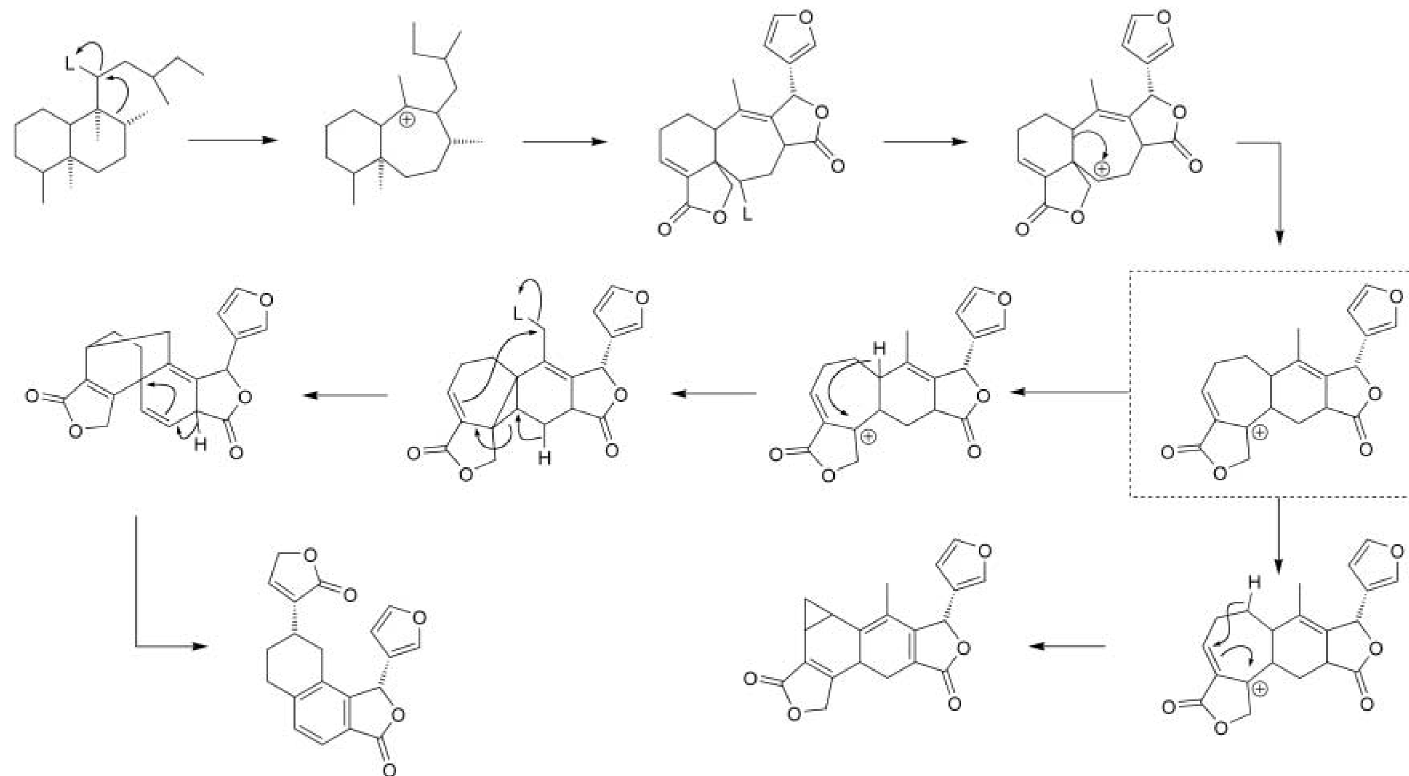
It is interesting that all the above diterpeniods were isolated from the same plant. The diterpenoids from *S. dugesii* are clerodanic diterpenoids, or diterpenoids with rearranged skeletons biogenetically related to a normal clerodane precursor. Their



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 *Calosphace*.

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}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}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 *Calosphace* [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. *^{1}H- 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}H,^{1}H)	HMBC (^{1}H,^{13}C)
H_a–C(1)	29.8 (*t*)	2.92 (*m*)	H_b–C(1), H_a–C(2), H_b–C(2)	C(2), C(3), C(5)
H_b–C(1)		2.05 (*m*)	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 (*t*)	2.48 (*m*)	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 (*m*)	H_a–C(1), H_b–C(1), H_a–C(2), H–C(3)	C(1), C(10)
H–C(3)	136.3 (*d*)	7.07 (*dd*, *J* = 2.4, 6.2)	H_a–C(2), H_b–C(2)	C(1), C(2), C(5), C(18)
C(4)	135.5 (*s*)			
C(5)	46.9 (*s*)			
H_a–C(6)	35.3 (*t*)	2.04 (*m*)	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 (*m*)	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 (*t*)	2.70 (*m*)	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 (*m*)	H_a–C(6), H_b–C(6), H_a–C(7)	C(5)
C(8)	127.5 (*s*)			
C(9)	124.5 (*s*)			
C(10)	147.3 (*s*)			
C(11)	159.7 (*s*)			
H–C(12)	75.5 (*d*)	6.23 (br. *s*)		C(8), C(11), C(13), C(14)
C(13)	121.6 (*s*)			
H–C(14)	109.0 (*d*)	6.52 (br. *s*)	H–C(15), H–C(16)	C(13), C(15), C(16)
H–C(15)	144.5 (*d*)	7.60 (br. *s*)	H–C(14), H–C(16)	C(13), C(14), C(16)
H–C(16)	142.2 (*d*)	7.80 (br. *s*)	H–C(14), H–C(15)	C(13), C(14), C(15)
C(17)	167.3 (*s*)			
C(18)	168.4 (*s*)			
H_a–C(19)	71.1 (*t*)	4.82 (*d*, *J* = 7.2)	H_b–C(19)	C(4), C(5), C(6), C(18)
H_b–C(19)		3.70 (*d*, *J* = 7.2)	H_a–C(19)	C(4), C(5),
H–C(20)	16.5 (*q*)	1.89 (*s*, 3 H)		C(9), C(10), C(11)

Comparison of the ^{1}H-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 $^{1}H,^{1}H$-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 (2*dd*, $J = 7.2$, 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 (2*m*, 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 $C_{20}H_{14}O_5$, 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 form *salvia puberula* [3].

Table 2. *^{1}H- 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 ($^{1}H,^{1}H$)	HMBC ($^{1}H,^{13}C$)
H_a−C(1)	29.7 (*t*)	3.17 (*dd*, $J = 7.0$, 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 (*dd*, $J = 7.0$, 13.6)	H_a−C(1), H−C(2)	C(2), C(3), C(6), C(9), C(10)
H−C(2)	128.5 (*d*)	5.98 (*td*, $J = 7.0$, 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 (*d*)	6.52 (*d*, $J = 9.6$)	H−C(2)	C(2), C(4), C(5), C(18)
C(4)	126.5 (*s*)			
C(5)	155.4 (*s*)			
C(6)	132.2 (*s*)			
H−C(7)	124.1 (*d*)	7.78 (br. *s*)		C(5), C(6), C(11), C(17)
C(8)	121.9 (*s*)			
C(9)	131.0 (*s*)			
C(10)	141.7 (*s*)			
C(11)	150.4 (*s*)			
H−C(12)	75.0 (*d*)	6.43 (br. *s*)		C(8), C(9), C(11), C(13), C(14), C(16), C(17)
C(13)	120.6 (*s*)			
H−C(14)	108.6 (*d*)	6.04 (br. *s*)	H−C(15)	C(12), C(13), C(15)
H−C(15)	144.3 (*d*)	7.35 (br. *s*)	H−C(14), H−C(16)	C(13), C(14), C(16)
H−C(16)	142.2 (*d*)	7.51 (br. *s*)	H−C(15)	C(13), C(14), C(15)
C(17)	169.3 (*s*)			
C(18)	172.5 (*s*)			
H_a−C(19)	69.8 (*t*)	5.39 (*d*, $J = 17.5$)	H_a−C(19)	C(4), C(5), C(18)
H_b−C(19)		5.34 (*d*, $J = 17.5$)	H_b−C(19)	C(4), C(5), C(18)
H−C(20)	15.5 (*q*)	2.24 (br. *s*, 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 $CH_2(3)$ group in **3** was 'flipped', now corresponding to $CH_2(1)$ in **2** (double-bond shift). The observed difference in the chemical shift of the $CH_2(1)$ 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 (2*dd*, $J = 7.0$, 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 $CH_2(1)$ 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 (2*dd*, $J = 6.6$, 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 g), **6** (55 mg), and **7** (5 mg).

*(8*R*,12a*S*)-8-(Furan-3-yl)-5,6,11,12-tetrahydro-7-methyl-3*H*-furo[3′,4′:4,5]cyclohepta[1,2-*d*][2]benzofuran-3,10(8*H*)-dione* (*Dugesin A*; **1**). White amorphous powder. $[\alpha]_D^{25} = +17.24$ ($c = 0.145$; 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. ^{1}H- 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 ($C_{20}H_{18}O_5^+$; calc. 338.115).

*(8*R*)-8-(Furan-3-yl)-6,8-dihydro-7-methyl-3*H*-furo[3′,4′:3,4]cyclohepta[1,2-*f*][2]benzofuran-3,10(1*H*)-dione* (*Dugesin B*; **2**). Colorless crystals (acetone). M.p. 220–223° (acetone). $[\alpha]_D^{19} = -123.08°$ ($CHCl_3$; $c = 0.325$). 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. ^{1}H- 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: 334.095 ($C_{20}H_{14}O_5^+$; calc. 344.084).

*(8*R*)-8-(Furan-3-yl)-4,8-dihydro-7-methyl-3*H*-furo[3′,4′:3,4]cyclohepta[1,2-*f*][2]benzofuran-3,10(1*H*)-dione* (isosalvipuberulin; **3**). Colorless crystals (acetone). ^{13}C-NMR ($CDCl_3$): 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)).

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