New Epoxydammarane Triterpenes from *Salvia santolinifolia*

by **Zaheer Ahmad**[a]), **Itrat Fatima**[b]), **Sajid Mehmood**[c]), **Rehana Ifzal**[a]), **Abdul Malik***[b]), and **Nighat Afza**[c])

[a]) Department of Chemistry, University of Karachi, Karachi-75270, Pakistan
[b]) H. E. J. Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan (phone: +92-21-4824926; e-mail: abdul.malik@iccs.edu)
[c]) Pharmaceutical Research Centre, PCSIR, Laboratories Complex Karachi, Karachi-75280, Pakistan

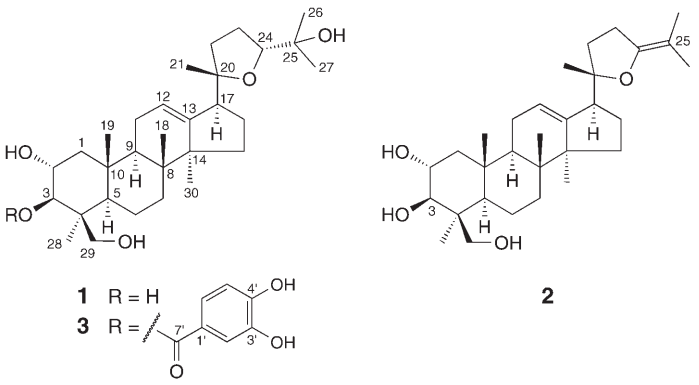
Three new 20,24-epoxydammarane triterpenes, santolins A–C (**1**–**3**), were isolated from the AcOEt-soluble fraction of the MeOH extract of *Salvia santolinifolia* (whole plant). Their structures were assigned based on ^{1}H-NMR, ^{13}C-NMR (DEPT), and 2D-NMR analyses, in combination with HR-MS experiments and comparison with literature data of related compounds.

Introduction. – The genus *Salvia* belongs to the family Labiatae, which comprises 24 species. Various species of this genus are widely used for the treatment of coronary heart diseases, particularly *angina pectoris*, myocardial infarction, amenorrhea, dysmenorrhea, and insomnia [1]. They also possess antiseptic, carminative diuretic, haemostatic, and spasmolytic activities [2].

Salvia santolinifolia is a branched, scabrous, and hispid straggling undershrub occurring in the rocky, arid areas of Pakistan. Its leaves and seeds are used as demulcent against diarrhea and hemorrhoids. The copious mucilage is frequently mixed and used with the seeds of *Plantago ovata* to produce '*ispaghol*'. The seeds are also said to be used in removing foreign bodies from the eye [3]. A literature survey of the genus *Salvia* revealed the presence of a variety of compounds, including diterpenes, aromatic ethers, phenolic glycosides, abietane-type pigments, and triterpenes [1][2]. Only two amyrin-type triterpenes have so far been reported from *S. santolinifolia* [4]. The ethnopharmacologic and chemotaxanomic importance of this genus prompted us to investigate the chemical constituents of *S. santolinifolia.* Herein, we report the isolation and characterization of three new 20,24-epoxydammarane triterpenes, santolins A–C (**1**–**3**).

Result and Discussion. – The MeOH extract of the whole plant was divided into fractions soluble in hexane, $CHCl_3$, AcOEt, BuOH, and H_2O. Column chromatography of the AcOEt-soluble fraction provided three new compounds, which showed positive *Liebermann–Burchard* test, indicating triterpene constituents.

Santolin A (**1**) was obtained as an amorphous powder. The IR spectrum showed the presence of OH groups (3360 cm^{-1}) and an olefin (1640 cm^{-1}). The molecular formula was deduced as $C_{30}H_{50}O_5$ through HR-FAB-MS, showing the $[M+H]^+$ peak at *m*/z 491.3736 (calc. 491.3736). The molecular formula was further confirmed by ^{13}C-NMR (DEPT) spectra (*Table 1*), which showed 30 signals: seven Me, nine CH_2, seven CH,



and seven quaternary C-atoms. The C=C bond was inferred from the signals at δ(C) 145.0 and 123.7, and three oxymethines were observed at δ(C) 70.0, 80.2, and 84.3. An oxygenated CH_2 was observed at δ(C) 65.6, together with two oxygenated quaternary C-atoms at δ(C) 81.2 and 72.1, respectively.

Table 1. *^{13}C-NMR Data of* **1**–**3**. At 100 MHz in $CDCl_3$; δ in ppm.

Position	**1**	**2**	**3**	Position	**1**	**2**	**3**
1	47.6	47.6	45.5	20	81.2	79.9	81.2
2	70.0	70.1	69.9	21	26.3	23.5	26.3
3	80.2	80.2	81.9	22	38.3	34.1	38.3
4	44.0	44.0	40.5	23	25.5	21.3	25.4
5	47.9	47.9	44.1	24	84.3	146.0	84.2
6	19.6	19.7	19.3	25	72.1	99.2	72.1
7	34.2	34.2	34.2	26	23.2	19.3	23.1
8	39.7	39.4	39.4	27	24.1	16.2	24.1
9	42.6	42.6	42.4	28	23.7	23.7	24.0
10	40.4	40.3	40.3	29	65.6	65.7	65.6
11	24.2	24.6	24.5	30	24.1	24.1	24.1
12	123.7	123.2	123.2	1′			130.9
13	145.0	145.0	145.1	2′			111.4
14	40.1	40.1	40.1	3′			148.1
15	34.6	34.6	34.6	4′			149.5
16	28.2	28.3	28.3	5′			123.7
17	41.3	40.5	41.3	6′			115.7
18	17.5	17.5	17.5	7′			175.0
19	17.7	17.7	17.6				

The ^{1}H-NMR spectrum of **1** (*Table 2*) showed signals for a trisubstituted C=C moiety (δ(H) 5.25 (*t*, *J* = 3.4 Hz, 1 H)). The three oxymethines resonated at δ(H) 3.72 (*ddd*, *J* = 9.8, 9.1, 3.5 Hz, 1 H), 3.70 (*t*, *J* = 11.0 Hz, 1 H), and 3.82 (*d*, *J* = 9.8 Hz, 1 H), and the oxymethylene groups were observed at δ(H) 3.39 (*d*, *J* = 10.9 Hz, 1 H) and 3.20 (*d*, *J* = 10.9 Hz, 1 H). There were seven Me *singlet*s at δ(H) 0.92, 0.85, 1.18, 1.17, 1.15,

Table 2. *^{1}H-NMR Data of* **1–3**. At 400 MHz in $CDCl_3$; δ in ppm, J in Hz.

Pos.	**1**	**2**	**3**
1	1.20–1.29 (*m*), 2.40–2.47 (*m*)	1.24–1.32 (*m*), 2.41–2.50 (*m*)	1.20–1.29 (*m*), 2.41–2.51 (*m*)
2	3.72 (*ddd*, *J* = 9.8, 9.1, 3.5)	3.70 (*ddd*, *J* = 9.8, 9.0, 3.4)	4.10 (*ddd*, *J* = 9.5, 9.1, 3.4)
3	3.82 (*d*, *J* = 9.8)	3.82 (*d*, *J* = 9.0)	4.89 (*d*, *J* = 9.1)
5	0.80–0.89 (*m*)	0.81–0.89 (*m*)	0.98–1.17 (*m*)
6	1.45–1.51 (*m*)	1.46–1.54 (*m*)	1.61–1.66 (*m*)
7	1.34–1.44 (*m*)	1.33–1.40 (*m*)	1.34–1.43 (*m*)
9	1.25–1.36 (*m*)	1.25–1.31 (*m*)	1.25–1.31(*m*)
11	2.09–2.11 (*m*)	2.08–2.15 (*m*)	2.08–2.14 (*m*)
12	5.25 (*t*, *J* = 3.4)	5.26 (*t*, *J* = 3.3)	5.24 (*t*, *J* = 3.3)
15	1.02–1.29 (*m*)	1.05–1.22 (*m*)	1.05–1.22 (*m*)
16	1.81–1.87 (*m*)	1.90–1.99 (*m*)	1.82–1.89 (*m*)
17	2.13–2.19 (*m*)	2.21–2.30 (*m*)	2.10–2.20 (*m*)
18	0.92 (*s*)	0.91 (*s*)	0.92 (*s*)
19	0.85 (*s*)	0.88 (*s*)	0.89 (*s*)
21	1.18 (*s*)	1.00 (*s*)	1.18 (*s*)
22	1.98–2.04 (*m*)	1.20–1.27 (*m*)	1.97–2.04 (*m*)
23	1.52–1.64 (*m*)	2.72–2.79 (*m*)	1.52–1.64 (*m*)
24	3.70 (*t*, *J* = 11.0)	–	3.71 (*t*, *J* = 10.5)
26	1.17 (*s*)	1.52 (*s*)	1.17 (*s*)
27	1.15 (*s*)	1.42 (*s*)	1.16 (*s*)
28	0.98 (*s*)	0.92 (*s*)	0.93 (*s*)
29	3.20 (*d*, *J* = 10.9), 3.39 (*d*, *J* = 10.9)	3.31 (*d*, *J* = 10.9), 3.48 (*d*, *J* = 10.9)	3.20 (*d*, *J* = 11.1), 3.48 (*d*, *J* = 11.1)
30	1.26 (*s*)	1.26 (*s*)	1.27 (*s*)
2′			7.12 (*d*, *J* = 1.5)
5′			6.72 (*d*, *J* = 8.1)
6′			6.90 (*dd*, *J* = 8.1, 1.5)

0.98, and 1.26. These data indicated that **1** was an epoxydammarane-type triterpene [5–9].

The EI mass spectrum of **1** showed a characteristic base peak at *m/z* 143, suggesting the presence of a methylated tetrahydrofuran group and a hydroxylated isopropyl side chain; thus, there were three remaining O-atoms and a trisubstituted C=C bond to be located. The oxymethine signal at δ(H) 3.82 (*d*) showed a ^{1}H,^{1}H-COSY correlation to another oxymethine at δ(H) 3.72 (*ddd*), and the latter, in turn, showed correlations to both δ(H) 2.40–2.47 (*m*, 1 H) and 1.20–1.29 (*m*, 1 H). Thus, the signals at δ(H) 3.82 and 3.72 were assigned to H–C(3) and H–C(2), respectively, as further confirmed through HMBC experiments (*Figure*). The larger coupling constants for both H–C(3) and H–C(2) allowed us to assign the equatorial configuration to the corresponding OH groups. The signals for CH_2(29) (δ(H) 3.39, 3.20) showed a ^2J correlation with C(4) at δ(C) 44.0, and ^3J correlations with C(3) (δ(C) 80.2), C(5) (δ(C) 47.9), and Me(28) (δ(C) 23.7), thereby confirming the presence of a CH_2OH group at C(4). Its configuration could be derived through ^{13}C-NMR experiments. In case of oxidation at C(29), Me(28) resonates at δ(C) 23–26; however, in case of oxidation at C(28), Me(29) is typically shifted upfield to δ(C) 14–16 [10]. Since the Me group attached to

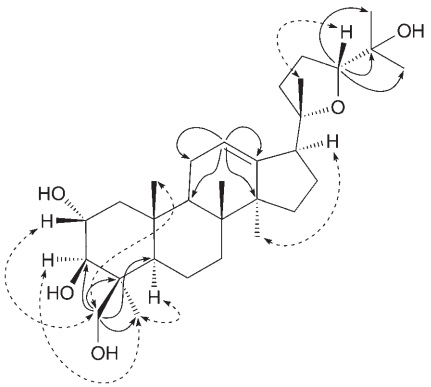


Figure. Key HMBC (H → C) *and NOESY* (← – – →) *correlations for* **1**

C(4) resonated at δ(C) 23.7, it had to be α-configured. The relative configurations at C(2), C(3), and C(4) could further be authenticated by NOESY correlations between the α-oriented Me(28) with both H–C(3) and H–C(5), as well as between the β-oriented Me(19) and both H–C(2) and H–C(29).

The presence of a hydroxyisopropyl moiety at C(24) was deduced by HMBC experiments (*Figure*). H–C(24) at δ(H) 3.70 showed a ^2J correlation with C(25) at δ(C) 72.1, and ^3J correlations with both C(26) and C(27). The remaining task was to ascertain the position of the C=C bond. Its possible loci were C(9) or C(12). Finally, the latter was confirmed through ^2J correlations of the olefinic signal at δ(H) 5.25 (H–C(12)) with both δ(C) 24.2 (C(11)) and 145.0 (C(13), as well as ^3J correlations with both δ(C) 42.6 (C(9)) and δ(C) 40.1 (C(14)).

The configuration at C(20) was assigned on the basis of the ^{13}C-NMR spectrum. It has previously been reported [11] that the chemical shift of Me(21) can be used to distinguish between the (20*R*) and (20*S*) configuration. In the case of (20*R*), Me(21) is more shielded, resonating at δ(C) 21–23, while it is shifted downfield at δ(C) 24–27 in the (20*S*) series. The observed chemical shift of Me(21) in case of **1**, δ(C) 26.3, thus indicated the (20*S*)-configuration. The configuration at C(24) was deduced by ^{1}H-NMR. The chemical shifts of δ(H) 3.70 (*t*, H–C(24)), 1.17 (*s*, Me(26)), and 1.15 (*s*, Me(27)) were compared with those reported for structurally related (20*S*,24*R*) and (20*S*,24*S*) compounds [11]. In the case of the (24*R*)-epimer, H–C(24) is observed as a *triplet* (*t*) at *ca.* δ(H) 3.70, and Me(26) and Me(27) show almost identical chemical shifts (within 0.03 ppm). On the other hand, in the (24*S*) epimer, H–C(24) is observed as a *doublet* of *doublet* (*dd*) at δ(H) 3.70, and Me(26) and Me(27) show significantly stronger variations in chemical shift. This allowed us to assign the (24*R*)-configuration to **1**. The *trans*-orientation of the substituents at C(20) and C(24) was further authenticated by a NOESY correlation between H–C(24) and Me(21). The chemical shifts of C(17) and H–C(17) were comparable to those of related epoxydammaranes, in accord with α-orientation of H–C(17), as further confirmed through a NOESY interaction between H–C(17) and Me(30).

Based on the above evidence, the structure of compound **1** was assigned as (2α,3β,20*S*,24*R*)-20,24-epoxydammar-12-ene-2,3,25,29-tetrol.

Compound **2** was obtained as an amorphous powder. Its IR spectrum was very similar to that of **1**. The molecular formula was deduced as $C_{30}H_{48}O_4$ by HR-EI-MS, which showed the M^+ peak at m/z 472.3552 (calc. 472.3553). The molecular formula was confirmed by ^{13}C-NMR, which showed 30 signals: seven Me, nine CH_2, six CH, and eight quaternary C-atoms. The ^{13}C-NMR (DEPT) spectrum of **2** (*Table 1*) was very similar to that of **1**, except for the absence of the aliphatic signals for C(24) and C(25), and the presence of signals due to an additional tetrasubstituted C=C bond at δ(C) 146.0 and 99.2. The ^{1}H-NMR spectrum of **2** (*Table 2*) was also similar to that of **1**, except for the absence of the oxygenated CH at δ(H) 3.70 and upfield-shifted Me(26) and Me(27) resonances.

The EI mass spectrum of **2** showed the base peak at m/z 125, suggesting that **2** differs from **1** by a dehydrated side chain. The presence of an additional C=C bond at C(24) was confirmed by ^2J correlations from both Me(26) and Me(27) to C(25) (δ(C) 99.2), and by ^3J correlations with C(24) (δ(C)146.0), respectively. From the NMR chemical shifts, coupling constants, and 2D-NMR spectra, the structure of **2** was assigned as (2α,3β,20*S*)-20,24-epoxydammar-12,24-dien-2,3,29-triol, and named santolin B.

Santolin C (**3**) was obtained as an amorphous powder. It's molecular formula was deduced as $C_{37}H_{54}O_8$ through HR-EI-MS, showing the M^+ peak at m/z 626.3818 (calc. 626.3819). The IR spectrum showed the presence of OH groups (3350 cm^{-1}), an ester function (1710 cm^{-1}), and a C=C bond (1650 cm^{-1}). The ^{13}C-NMR (DEPT) spectrum (*Table 1*) showed 37 signals: seven Me, nine CH_2, ten CH, and eleven quaternary C-atoms. Most of the ^{13}C-NMR signals of **3** were similar to those of **1**, except the additional signals due to a 3,4-dihydroxybenzoyl moiety [δ(C) 175.0, 149.5, 148.1, 130.9, 123.7, 115.7, 111.4]. The ^{1}H-NMR spectrum of **3** (*Table 2*) was also similar to that of **1**, except for additional peaks due the ester moiety [δ(H) 6.72 (*d*, J = 8.1 Hz, 1 H); 6.90 (*dd*, J = 8.1, 1.5 Hz, 1 H); 7.12 (*d*, J = 1.5 Hz, 1 H)]. The ester was attached at position 3, based on HMBC correlations; H–C(3) (δ(H) 4.89) showed ^2J correlations to both C(2) (δ(C) 69.9) and C(4) (δ(C) 40.5), as well as ^3J correlations with C(1) (δ(C) 45.5), C(5) (δ(C) 44.1), C(29) (δ(C) 65.6), C(28) (δ(C) 24.0), and the ester C=O group (δ(C) 175.0). From the NMR chemical shifts, coupling constants, and 2D-NMR spectra, the structure of compound **3** was, thus, assigned as (2α,3β,20*S*,24*R*)-20,24-epoxy-2,25,29-trihydroxydammar-12-ene-3-yl 3,4-dihydroxybenzoate.

Experimental Part

General. Column chromatography (CC): silica gel (230–400 mesh; *Merck*). Thin-layer chromatography (TLC): silica-gel *60 F_{254}* plates (*Merck*). Optical rotations: *Jasco DIP-360* digital polarimeter. IR Spectra: *Jasco 302-A* spectrophotometer, in $CHCl_3$ soln.; in cm^{-1}. NMR Spectra: *Bruker* instrument; δ in ppm, J in Hz. EI-, FAB-, and HR-EI-MS: *Jeol JMS-HX-110* and *JMS-DA-500* mass spectrometers; in m/z (rel. %).

Plant Material. The whole plant of *S. santolinifolia* Boiss. was collected from Karachi (Pakistan) in July 2002, and identified by Dr. *Surriya Khatoon*, Plant Taxonomist, Department of Botany, University of Karachi, where a voucher specimen (LS 831) was deposited.

Extraction and Isolation. The shade-dried, ground whole plant of *S. santolinifolia* (24 kg) was extracted with MeOH (3 × 50 l). The combined MeOH extracts were concentrated, and the residue (550 g) was divided into hexane-, $CHCl_3$-, AcOEt-, BuOH-, and water-soluble fractions. The AcOEt-soluble fraction (75 g) was subjected to CC (SiO_2; hexane/$CHCl_3$, $CHCl_3$, $CHCl_3$/MeOH of increasing

polarity) to afford four major fractions: *Fr. A – D. Fr. A*, eluted with $CHCl_3$/MeOH 95 : 5, was subjected to CC (SiO_2; $CHCl_3$/MeOH 89 : 11) to afford **3** (12 mg). *Fr. C*, eluted with $CHCl_3$/MeOH 91 : 9, was purified by CC (SiO_2; $CHCl_3$/MeOH 93 : 7), followed by separation of the resulting binary mixture by prep. TLC (SiO_2; $CHCl_3$/MeOH 88 : 12) to yield **1** (10 mg) and **2** (14 mg).

Santolin A (= *(2α,3β,20*S*,24*R*)-20,24-Epoxydammar-12-ene-2,3,25,29-tetrol*; **1**). Amorphous powder. M.p. 171–174°. $[\alpha]_D^{25} = +8.5$ ($c = 0.02$, MeOH). IR (KBr): 3360, 1640. ^{1}H- and ^{13}C-NMR: see *Tables 2* and *1*, resp. EI-MS: 475 (11, $[M - Me]^+$), 472 (62), 457 (42), 415 (45), 397 (50), 143 (100), 125 (22). HR-FAB-MS (pos.): 491.3736 ($[M + H]^+$, $C_{30}H_{51}O_5^+$; calc. 491.3732).

Santolin B (= *(2α,3β,20*S*)-20,24-Epoxydammar-12,24-dien-2,3,29-triol*; **2**). Amorphous powder. M.p. 220–225°. $[\alpha]_D^{25} = +11.0$ ($c = 0.02$, MeOH). IR (KBr): 3360, 1640. ^{1}H- and ^{13}C-NMR: see *Tables 2* and *1*, resp. EI-MS: 472 (9), 457 (22), 454 (10), 125 (100), 110 (75). HR-EI-MS: 472.3552 (M^+, $C_{30}H_{48}O_4^+$; calc. 472.3553).

Santolin C (= *(2α,3β,20*S*,24*R*)-20,24-Epoxy-2,25,29-trihydroxydammar-12-ene-3-yl 3,4-Dihydroxybenzoate*; **3**). Amorphous powder. M.p. 176–179°. $[\alpha]_D^{25} = +34.0$ ($c = 0.02$, MeOH). IR (KBr): 3350, 1710, 1650. ^{1}H- and ^{13}C-NMR: see *Tables 2* and *1*, resp. EI-MS: 626 (11), 611 (15), 608 (30), 473 (22), 413 (10), 395 (46), 143 (100), 125 (75), 77 (54). HR-EI-MS: 626.3818 (M^+, $C_{37}H_{54}O_8^+$; calc. 626.3819).

REFERENCES

[1] S. Y. Ryu, Z. No, S. H. Kim, J. W. Ahn, *Planta Med.* **1997**, *63*, 44.
[2] A. Ulubelen, S. Oksuz, U. Kolak, H. Biriman, W. Voelter, *Planta Med.* **2000**, *66*, 627.
[3] A. Krishnamurthi, 'The Wealth of India', CSIR New Delhi, 1978, Vol. 9, p. 195.
[4] S. Mehmood, N. Riaz, S. A. Nawaz, N. Afza, A. Malik, M. I. Choudhary, *Arch. Pharm. Res.* **2006**, *29*, 195.
[5] C. M. Hasan, A. Islam, M. Ahmad, P. G. Watermann, *Phytochemistry* **1984**, *23*, 2583.
[6] M. Nagai, N. Tanaka, O. Tanaka, S. Ichikawa, *Chem. Pharm. Bull.* **1973**, *21*, 2061.
[7] O. Tanaka, S. Yahara, *Phytochemistry* **1978**, *17*, 1353.
[8] M. M. Rao, H. Meshulam, R. Zelnik, D. Lavie, *Tetrahedron* **1975**, *31*, 333.
[9] P. G. Watermann, S. Ampofo, *Phytochemistry* **1985**, *24*, 2925.
[10] K. Amimoto, K. Yoshikawa, S. Arihara, *Phytochemistry* **1993**, *33*, 1475.
[11] T. Hoshino, Y. Yonemura, T. Abe, Y. Sugino, *Org. Biomol. Chem.* **2007**, *5*, 792.

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