HETEROCYCLES, Vol. 68, No. 1, 2006, pp. 159 – 165. © The Japan Institute of Heterocyclic Chemistry
Received, 15th September, 2005, Accepted, 11th November, 2005, Published online, 15th November, 2005. COM-05-10565

NEW ABIETANE DITERPENE ALKALOIDS POSSESSING AN OXAZOLE RING FROM *SALVIA TRIJUGA*

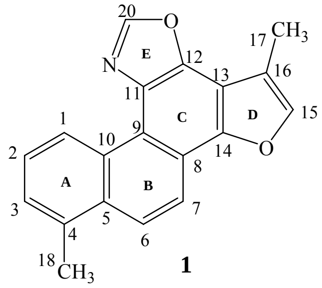
Fu-Wen Lin,[a] Amooru G. Damu,[a] and Tian-Shung Wu[a,b]*

[a]Department of Chemistry, National Cheng Kung University, Tainan 701, Taiwan, R.O.C.
[b]National Research Institute of Chinese Medicine, Taipei 112, Taiwan, R. O. C.
Tel: 886-6-2747538, Fax: 886-6-2740552, E-mail: tswu@mail.ncku.edu.tw

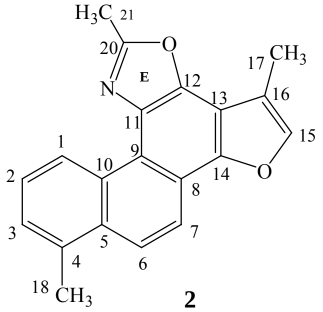
Abstract –Two new diterpene alkaloids, isosalviamine-A (**1**) and B (**2**) and nine known tanshinones (**3-11**) were isolated from the MeOH extract of roots of *S. trijuga*. The structures of these new compounds were elucidated from ^{1}H and ^{13}C NMR spectra with the aid of COSY, NOESY, HMQC and HMBC experiments.

INTRODUCTION

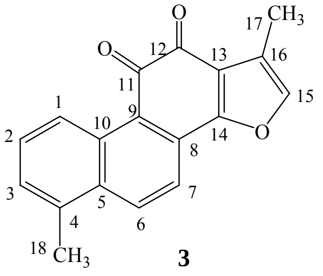
A member of the Lamiaceae family, the genus *Salvia* consists of some 500 species found worldwide, 84 of which were distributed in China. Since ancient times, many species of this genus have been credited with medicinal properties and thus merit investigation.[1-4] Some *Salvia* extracts have shown interesting biological activity such as antibacterial,[5] antiplasmodial,[6] antituberculosis,[7] antiphlogistic,[7] cardioactive,[5] antidiabetic,[7] anti-inflammatory,[8] analgesic,[8] antipyretic,[8] antispasmodic,[8] antitumor,[9] antiviral,[10] hallucinogenic,[11] trypanocidal,[12] antifungal,[13] and antioxidant[14] agent and the active constituents of these species are thought to be abietane diterpenes.[15, 16] *Salvia trijuga* Diels is a perennial herb distributed widely in the southern China.[3] The root of this plant has been used as a substitute for “Danshen” (root of *S. miltiorrhiza Bunge*) a traditional Chinese medicine which has reputed therapeutic effects in the treatment of menstrual disorders, [1] menostatis, [1] menorrhalgia, [1] insomnia, [17] arthritis, [17] and coronary heart and cerebrovascular diseases[18] as well as neurasthenic insomnia.[18] Thorough literature survey showed that there were few reports on the isolation of tritepenes and tanshinones from the titled plant.[19-23] Our detailed chemical investigation on the roots of *S. trijuga* has yielded 11 compounds, out of which **1** and **2** were



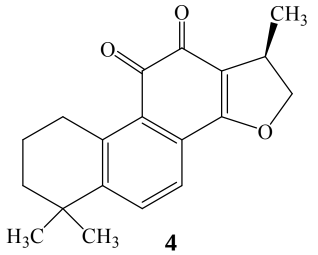
1



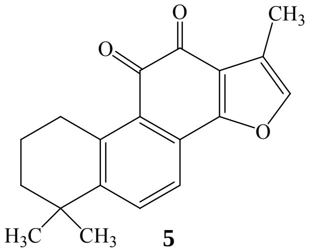
2



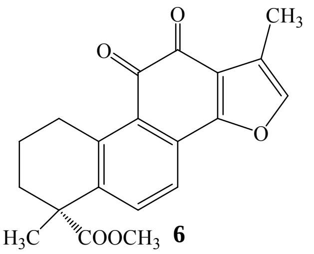
3



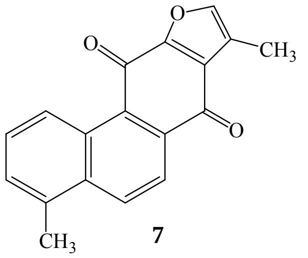
4



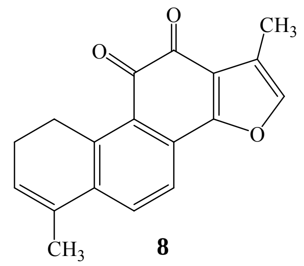
5



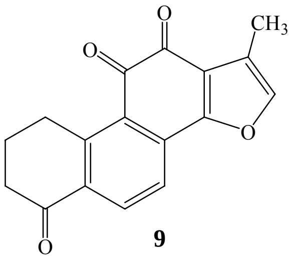
6



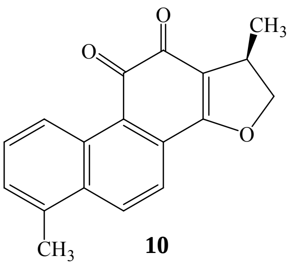
7



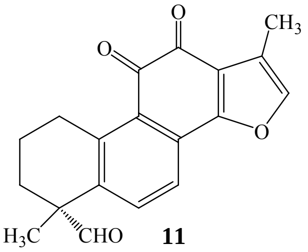
8



9



10



11

new nitrogen containing abietane diterpenes. This paper describes the isolation and identification of these two new abietane diterpene alkaloids, isosalviamine-A (**1**) and isosalviamine-B (**2**).

RESULTS AND DISCUSSION

The concentrated MeOH extract of the root of *S. trijuga* was a partitioned into $CHCl_3$ and H_2O solubles. A series of chromatographic separation of the $CHCl_3$ solubles resulted in the isolation of 9 known tanshinones and two new compounds whose structures (**1**) and (**2**) were elucidated on the basis of spectroscopic evidence as follow.

Isosalviamine A (**1**) was isolated as a yellow powder. The HREIMS exhibited a molecular ion at *m/z* 287.0944 compatible with the molecular formula $C_{19}H_{13}NO_2$. This was in good agreement with the 19 carbon atom resonances observed in the ^{13}C NMR spectrum. These 19 carbon signals were assigned to two methyls, seven methines, and ten quaternary carbons. The ^1H NMR spectrum (Table 1) showed an AMX spin system for three consecutive protons at δ10.26 (1H, d, J = 8.4 Hz), 7.69 (1H, dd, J = 8.4, 7.2 Hz), and 7.51 (1H, d, J = 7.2 Hz), a pair of *ortho* coupled doublets at δ 8.16 (1H, d, J = 8.8 Hz)and 8.41(1H, d, J = 8.8 Hz), a quartet at δ 7.66 (1H, q, J = 1.2 Hz) and a methyl doublet at δ 2.59 (1H, d, J =

1.2 Hz) assignable to methylated furan ring, and a methyl at δ 2.83 (1H, s). These ^{1}H and ^{13}C NMR spectral data were almost identical with the literature values of tanshinone I (**3**), a principal constituent of *Salvia* species.[24] However, **1** showed different ^{1}H and ^{13}C NMR spectral data from those of tanshinone I in the signals of H-1, C-11, C-12 and appearance of one more proton and its carbon. Absence of two carbonyl signals and the appearance of an extra proton at δ_H 8.36 (s) and its corresponding carbon at δ_C 149.7 together with characteristic IR absorptions at 1527 and 819 cm^{-1} for O-C=N group indicated that orthoquinone group in tanshinone I was replaced by an oxazole ring in **1**. This was also supported by the prominent fragment peaks at *m/z* 244 due to [M-NCHO]$^+$ ions. Recently, Don *et al.* reported nitrogen

Table 1. ^{1}H and ^{13}C NMR spectral data of compounds (**1**-**2**) (in $CDCl_3$, 400 MHz, TMS as int. stand.)

H	**1**	**2**	**3**	C	**1**	**2**
1	10.26 d (8.4)	10.27 d (8.4)	9.24 d (8.8)	1	126.0	126.1
2	7.69 dd (8.4, 7.2)	7.68 dd (8.4, 7.2)	7.54 dd (8.8, 7.2)	2	126.6	126.6
3	7.51 d (7.2)	7.49 d (7.2)	7.33 d (7.2)	3	127.6	127.6
6	8.16 d (8.8)	8.12 d (8.8)	8.29 d (8.8)	4	134.4	134.2
7	8.41 d (8.8)	8.39 d (8.8)	7.80 d (8.8)	5	130.1	131.0
15	7.66 q (1.2)	7.64 q (1.6)	7.29 q (1.6)	6	118.8	118.4
17	2.59 d (1.2)	2.57 d (1.6)	2.28 d (1.6)	7	122.9	122.4
18, 19	2.83 s	2.82 s	2.68 s	8	117.3	116.8
20	8.36 s			9	120.1	119.7
21		2.86 s		10	129.7	130.5
				11	130.9	133.3
				12	149.0	143.1
				13	112.4	112.1
				14	151.1	150.5
				15	141.8	141.6
				16	114.9	114.7
				17	9.2	9.3
				18, 19	20.2	20.1
				20	149.7	160.8
				21		14.8

containing compounds from *S. miltiorrhiza* and explained the orientation nitrogen and oxygen atoms of oxazole ring on the basis of C-11 and 12 chemical shift values and semisynthesis.[25] Thus while N atom connected to C-12 in the oxazole ring of abietane diterpene alkaloids for example, neosalvianen, C-12 and C-11 resonate at δ 132.6 and 144.4, respectively whereas abietane alkaloids with opposite orientation in oxazole ring (ex. salvianen and salvianan) exhibit C-12 and C-11 resonanances in the range of δ 145-148 and 130-135. Since the C-11 (130.9) and C-12 (149.0) resonances of **1** matched well with those of salvianen and salvianan, the N atom in the oxazole ring was attached to C-11 and thus O atom to C-12. This orientation also supported by an extreme downfield shift of H-1 to δ 10.26 due to C=N bond close to H-1. From these spectral analyses, the structure (**1**) was assigned for isosalviamin-A.

Isosalviamine-B was obtained as pale yellow needles and showed the $[M]^+$ ion at *m/z* 301.1110 (HREIMS) in agreement with the molecular formula $C_{20}H_{15}NO_2$. Its IR spectrum featured strong absorptions at 1521 and 817 cm^{-1} due to oxazole ring. The ^1H and ^{13}C NMR spectral data of **2** were very similar with those of **1** except H- and C-20 signals of **1** that were replaced by those of a methyl group (δ_H 2.86 and δ_C 14.8) and a quaternary carbon (δ 160.8), respectively. Therefore, from these comparison the structure of **2** was readily apparent as 21-methyl derivative of isosalviamine-A, and named as isosalviamine-B. The proposed structure was also supported by the prominent ions at *m/z* 286, 244 due to $[M-CH_3]^+$, $[M-CH_3-NCHO]^+$ ions. The full assignment of the ^1H and ^{13}C NMR spectral resonances of **1** and **2** was made using COSY, NOESY, HMQC, and HMBC spectral experiments.

Other previously known tanshinone diterpenes isolated from the roots of this plant were identified as tanshinone I (**3**),[24] cryptotanshinone (**4**),[24] tanshinone IIA (**5**),[24] methyltanshinoate (**6**),[26] isotanshinone I (**7**),[27] 1,2-dihydrotanshinone (**8**),[28] nortanshinone (**9**),[26] dihydrotanshinone I (**10**)[24] and dehydrotanshinaldehyde (**11**) [26] by the comparison of their spectral data with those of reported in the literature.

EXPERIMENTAL

General Experimental procedures. Melting points were determined on Yanaco MP-S3 micro-melting point apparatus without correction. UV spectra were taken on a Hitachi UV-3210 spectrophotometer. IR spectra were recorded on a Shimadzu IR Prestige-21 spectrophotometer as KBr discs. ^1H, ^{13}C, COSY, HMQC, HMBC, and NOESY NMR spectra were recorded on the Varian Unity Plus 400 NMR spectrometer, using tetramethylsilane (TMS) as the internal standard, and all chemical shifts were reported in parts per million (ppm, δ). All the MS and HRMS (EI) spectra were obtained on a VG-70-250S mass spectrometer.

Plant Material. The roots of *Salvia trijuga* were collected in Li Jiang, Yunnan Province, People's Republic of China in Sepertember, 1997 by Miss S. Zhang and identified by Professor C. S. Kuoh. A voucher specimen (TSWu 97201) has been deposited in the Herbarium of the National Cheng Kung University, Tainan, Taiwan.

Extraction and Isolation. The dried roots of *S. trijuga* (9.3 kg) were extracted with MeOH (20 L) under reflux for 8 h, (for six times) and concentrated under reduced pressure to afford dark brown extract (1.2 kg). The extract was partitioned with $CHCl_3/H_2O$ (1:1) into $CHCl_3$ and H_2O layers. The $CHCl_3$ solubles were concentrated under reduced pressure to give brown syrup (144 g) which was column chromatographed over silica gel by eluting with hexane/acetone (19:1) gradients by increasing proportions of acetone (5%→100%) to give 8 fractions.

Fraction 1 was re-chromatographed over silica gel and eluted with n-hexane/ethyl acetate (49:1) to afford pure **1** (0.56 mg). Fraction 2 on rechromatography over a silica gel by eluting with n-hexane/ethyl acetate (19:1) gave **2** (0.53 mg). Fractions 3 and 4 were combined and rechromatographed over silica gel by eluting with hexane/ethyl acetate (9:1) to afford pure **7** (1.19 mg). A series of chromatographic separations of fraction 5 with hexane/chloroform mixtures yielded pure **3** (32.5 g), **5** (23.5 g), and **8** (1.03 mg). Fraction 6 subjected to a series of chromatographic separations over silica gel with hexane/chloroform mixtures to afford pure **4** (15.21 g) and **6** (4.59 mg). Fraction 7 was further column chromatographed over silica gel with chloroform/methanol (19:1) to give pure **9** (1.05 mg), **10** (8.2 mg) and **11** (3.85 mg).

Isosalviamine A (1): $C_{19}H_{13}NO_2$: Yellow amorphous powder (acetone), mp: 178-180℃; UV λ_{max} (MeOH) (log ε) nm: 359 (2.57), 342 (2.64), 326 (2.55), 311 (sh) (3.11), 303 (3.47), 290 (sh) (3.53), 282 (sh) (3.80), 276 (4.00), 267 (3.93), 258 (sh) (3.74), 239 (3.61); IR (KBr) ν_{max} cm^{-1}: 1640, 1527, 1470, 1393, 1308, 819, 763; ^1H NMR ($CDCl_3$, 400 MHz); ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 1; EIMS *m/z* (*rel. int.* %): 287 (100, M^+), 272 (10), 258 (7), 244 (4), 230 (3), 216 (3), 125 (15), 111 (24), 55 (51); HR-EIMS *m/z* 287.0944 (M^+) (calcd for $C_{19}H_{13}NO_2$, 287.0946).

Isosalviamine B (2): $C_{20}H_{15}NO_2$: pale yellow needles (acetone) mp: 175-177℃; UV λ_{max} (MeOH) (log ε) nm: 361 (2.15), 310 (sh) (2.91), 303 (3.2), 289 (sh) (3.35), 283 (sh) (3.49), 276 (3.66), 267 (sh) (3.63), 259 (3.47); IR (KBr) ν_{max} cm^{-1}: 1731, 1619, 1596, 1521, 1462, 1386, 1168, 817, 765; ^1H NMR ($CDCl_3$, 400 MHz); ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 1; EIMS *m/z* (*rel. int.* %): 301 (100, M^+), 286 (51), 272 (15), 258 (10), 244 (10), 202 (10), 150 (19), 57 (21); HR-EIMS *m/z* 301.1110 (M^+) (calcd for $C_{20}H_{15}NO_2$, 301.1102)

ACKNOWLEDGEMENTS

The present address of Fu-Wen Lin is Food Industry Research and Development Institute in Taiwan, from

National Cheng Kung University, Tainan, Taiwan, in 2002. The authors are grateful for financial support from the National Science Council, Taiwan, Republic of China (NSC 89-2314-B006-061) awarded to T. S. Wu and also thankful to the National Research Institute of Chinese Medicine, Taiwan, Republic of China, for partial financial support of this research. We are thankful to Miss S. Zhang (Institute of Materia Medica, Chinese Academy of Medicinal Sciences, Beijing, People's Republic of China) for plant collection and Professor C. S. Kuoh (National Cheng Kung University, Tainan, Taiwan) for plant identification.

REFERENCES

1. H. M. Chang and P. P. H. But, "Pharmacology and Applications of Chinese Materia Medica", World Scientific Publishing, Singapore, 1986, Vol.1, p. 255.
2. Y. Lu and L. Yeap Foo, *Phytochemistry*, 2002, **59**, 117.
3. K. Bergwein, *Am. Perfum. Cosmet.*, 1968, **83**, 41.
4. Pharmacology research institute of Chinese medicine academy, 2nd, People Hygiene Publishing Co., Beijing, 1979, Vol. 1, pp. 339-349.
5. A. Ulubelen, *Phytochemistry*, 2003, **64**, 395.
6. M. Sairafianpour, J. Christensen, D. Staerk, B. A. Budnik, A. Kharazmi, K. Bagherzadeh, and J. W. Jaroszewski, *J. Nat. Prod.*, 2001, **64**, 1398.
7. A. Ulubelen, G. Topen, and C. B. Johansson, *J. Nat. Prod.*, 1997, **60**, 1275.
8. M. Hernandez-Perez, R. M. Rabanal, M. C. De la Torre, and B. Rodrıguez, *Planta Med.*,1995, **61**, 505.
9. X. Wang, K. F. Bastow, C. M. Sun, Y. L. Lin, H. J. Yu, M. J. Don, T. S. Wu, S. Nakamura, and K. H. Lee, *J. Med. Chem.,* 2004**, 47***,* 5816.
10. M. Tada, K. Okuno, K. Chiba, E. Ohnishi, and T. Yoshi, *Phytochemistry*, 1994, **35**, 539.
11. L. J. Valdes, H. M. Chang, D. C. Visger, and M. Koreeda, *Org. Lett.*, 2001, **3**, 3935.
12. N. Uchiyama, F. Kiuchi, M. Ito, G. Honda, Y. Takeda, O. K. Khodzhimatov, and O. A. Asurmetov, *J. Nat. Prod.*, 2003, **66**, 128.
13. D. Pitarokili, M. Colaudis, N. Petsikos-Panayotarou, and O. Tzakou, *J. Agric. Food Chem.*, 2002, **50**, 6688.
14. M. E. Cuvelier, C. Berset, and H. Richard, *J. Agric. Food Chem.*, 1994, **42**, 665.
15. H. L. Rodriguez, B. Esquivel, J. Cardenas, and T. Ramamoorthy, The Distribution of Diterpenoids in *Salvia*. In *Advances in Labiatae Science*, ed. by R. M. Harley and T. Reynolds, Royal Botanic Gardens, Kew, U.K., 1992, p. 335.
16. B. M. Fraga, C. E. Diaz, A. Guadano, and A. G. Coloma*, J. Agric. Food Chem.*, 2005**, 53**, 5200.

17. Jiangsu New Medical College *Zhong Yao Da Ci Dian (Dictionary of Chinese Material Medica)*; Shanghai Scientific and Technological Publishers, Shanghai, 1988, pp. 478-482.
18. W. Z. Chen, *Acta Pharm. Sin. (Yao Xue Xue Bao)*, 1984, **19**, 876.
19. B. J. Yang, X. L. Huang, Z. B. Hu, and Z. X. Chen, *Yao Xue Xue Bao*, 1982, **17**, 517.
20. X. Z. Lu, H. W. Luo, J. Ji, and H. Cai, *Yao Xue Xue Bao*, 1991, **26**, 193.
21. X. Lu and H. Luo, *Zhongguo Zhong Yao Za Zhi*, 1996, **21**, 424.
22. R. Kasimu, K. Tanaka, Y. Tezuka, Z. N. Gong, J. X. Li, P. Basnet, T. Namba, and S. Kadota, *Chem. Pharm. Bull.*, 1998, **46**, 500.
23. S. P. Ip, H. Yang, H. D. Sun, and C. T. Che, *Planta Med.*, 2002, **68**, 1077.
24. G. Honda, Y. Koezuka, and M. Tabata, *Chem. Pharm. Bull.*, 1988, **36**, 408.
25. M. J. Don, C. C. Shen, Y. L. Lin, W. J. Syu, Y. H. Ding, and C. M. Sun, *J. Nat. Prod.*, 2005, **68**, 1066.
26. J. Lee and J. K. Snyder, *J. Org. Chem.*, 1990, **55**, 4995.
27. Y. Inouye and H. Kakisawa, *Bull. Chem. Soc. Jpn.*, 1969, **42**, 3318.
28. S. Y. Ryu, C. O. Lee, and S. U. Choi, *Planta Med.*, 1997, **63**, 339.