PERGAMON

Phytochemistry 50 (1999) 1411–1415

PHYTOCHEMISTRY

Alkaloids and other constituents from *Tribulus terrestris*

Tian-Shung Wu[a,*], Li-Shian Shi[b], Shang-Chu Kuo[b,c]

[a] *Department of Chemistry, National Cheng Kung University, Tainan, Taiwan, R.O.C.*
[b] *Graduate Institute of Pharmaceutical Chemistry, China Medical College, Taichung, Taiwan, R.O.C.*
[c] *National Research Institute of Chinese Medicine, Taipei, Taiwan, R.O.C.*

Received in revised form 20 October 1997

Abstract

Three new compounds, terrestribisamide, 25*R*-spirost-4-en-3,12-dione and tribulusterine, together with 10 known compounds, *N*-*p*-coumaroyltyramine, terrestriamide, hecogenin, aurantiamide acetate, xanthosine, fatty acid ester, ferulic acid, vanillin, *p*-hydroxybenzoic acid and *β*-sitosterol, were isolated and characterized from dried fruits of *Tribulus terrestris*. Structures of these compounds were determined by spectral analysis. © 1999 Elsevier Science. All rights reserved

Keywords: *Tribulus terrestris*; Zygophyllaceae; fruits; amide; steroid; alkaloid.

1. Introduction

"Jili", the fruits of *Tribulus terrestris*, is a famous traditional Chinese medicine. In the Shern-Nong Pharmacopoeia (the oldest known pharmacological work in China), it is described as a highly valuable drug used to restore the depressed liver for the treatment of fullness in the chest and mastitis and also used to dispel the wind and clear the eyes for the treatment of acute conjunctivitis, headache and vertigo (Xie & Huang, 1988). *Tribulus terrestris* is also reported to have antimicrobial, antihypertension, diuretic, antiacetylcholine and haemolytic activity and to stimulate spermatogenesis and libido (Jit & Nag, 1986; Bose, Saifi, Vijayvargia, & Bhatnager, 1964; Tomova, 1988; Sharma, Norula, & Varadarajan, 1977). Alkaloids, steroids, flavonoids and carbohydrates have been isolated from this plant (Duan & Zhou, 1993; Chiang, 1977; Mahato, Nianjan, Ganguly, Miyaharo, & Kawasaki, 1981; Saleh & El-Hadidi, 1982; Saleh, Ahmed, & Abdalla, 1982; Mahato, Shau, Pal, Chakravarti, Chakravaryi, & Ghosh, 1978; Perepelitsa & Kintya, 1975; Tomova, Gyulemetova, Zarkova, Peeva, Pangarova, & Simova, 1981; Zafar, Lalwani, & Siddiqui, 1989; Chiu & Chang, 1986; Nag, Mathur, & Goyal, 1979; Mahato, Niranjan, Ganguly, Miyaharo & Kawasaki, 1981; Mathur, Nag, & Goyal, 1977; Vasi & Kalintha, 1982).

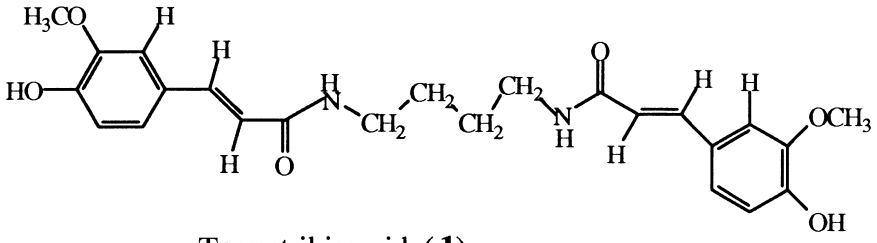
During our continuing search for novel bioactive natural products, a crude extract of the dried fruits of *T. terrestris* showed significant anti-inflammatory and immunosuppressive activities. This led us to reinvestigate its constituents. We report here the isolation and structural elucidation of three new compounds and ten known compounds from methanolic extracts of the dried fruits.

2. Results and discussion

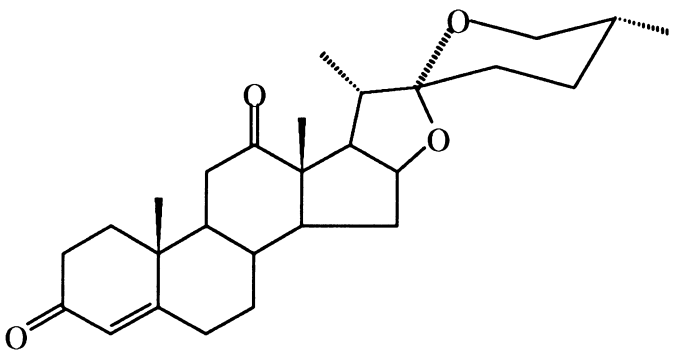
Terrestribisamide (**1**) was obtained as colourless powder, and its HRFAB mass spectrum indicated a pseudomolecular formula $C_{24}H_{29}N_2O_6$. The UV absorptions at 319, 294, 234 and 220 nm were similar to terrestriamide (**5**), being characteristic of 3,4-dioxygenated cinnamic acid derivatives (Ren, Chen, Yang, & Zhu, 1994). The presence of hydroxyl and amide groups was inferred by IR absorption bands at 1652 and 3490 cm^{-1} correlated with two D_2O-exchangeable signals at δ 7.99 (2H, *t*, $J = 5.2$ Hz) and 9.4 (2H, *s*) in ^1H NMR spectrum. The ^1H NMR spectrum of **1** showed the presence of a sharp singlet signal at δ 3.78 (6H), due to the methoxyl group and methylene groups at δ 1.45 (4H, *m*) and δ 3.14 (4H, *m*). The presence of disubstituted cinnamate groups was confirmed by ABX-type signals at δ 6.76 (2H, *d*, $J = 8.0$ Hz), 6.96 (2H, *dd*, $J = 8.0$, 1.5 Hz) and 7.09 (2H, *d*, $J = 1.5$ Hz), together with olefinic protons at δ 6.40 (2H, *d*, $J = 15.6$ Hz) and 7.29(2H, *d*, $J = 15.6$ Hz) attributable to H-7, 7′, 5, 5′, 6, 6′, 2, 2′ and 8, 8′, respectively. The NOESY spectrum (Fig. 1) of **1** showed a cross-peak between the methoxyl group at δ 3.78 and H-2, 2′ at δ 7.09, indicating that the methoxyl group was substituted at C-3, 3′. These data were in agreement with those for

* Corresponding author.

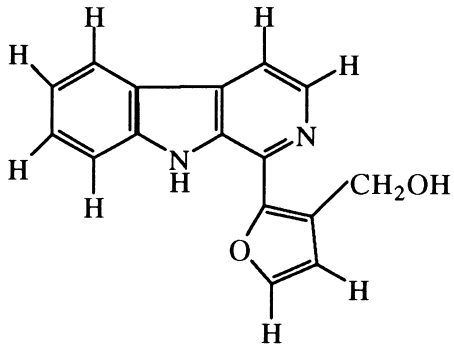
PII: S0031–9422(97)01086–8



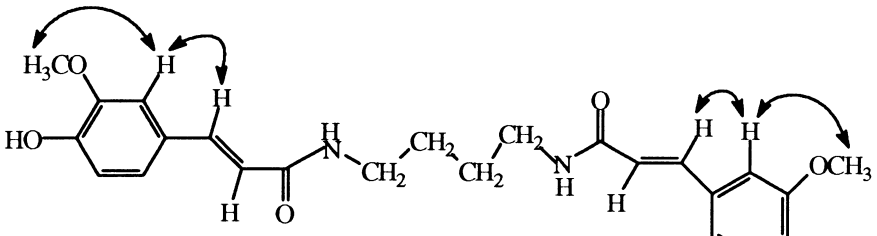
Terrestribisamide(**1**)



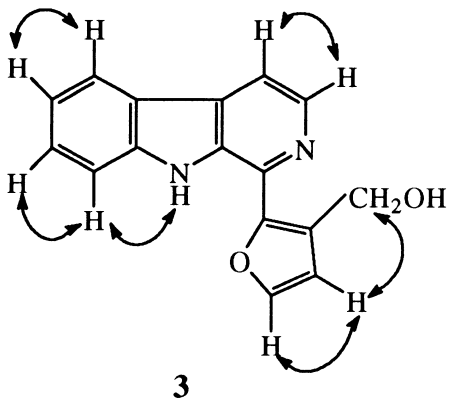
25*R*-Spirost-4-en-3,12-dione(**2**)



Tribulusterine(**3**)



1



3

Fig. 1. NOESY correlations for compounds **1** and **3**.

the FAB mass spectrum which showed a parent peak at m/z 441 and a fragment ion at m/z 221. Based on these results, we assigned structure **1** to terrestribisamide.

25*R*-Spirost-4-en-3,12-dione (**2**) was isolated as an optically active colourless powder. The molecular formula $C_{27}H_{38}O_4$ was determined by high resolution mass spectrometry. A positive reaction with the Leibermann-Bürchard reagent, together with its molecular formula, indicated that **2** was a steroid. The UV spectrum with an absorption maximum at 238 nm was characteristic of an α, β-unsaturated ketone system in the molecule. The IR spectrum exhibited absorption bands at 1706 and 1674 cm^{-1} due to carbonyl and α, β-unsaturated ketone, respectively. Compound **2** has four hydrogen atoms less than hecogenin (**6**) by comparison of their mass spectra. The ^1H and ^{13}C NMR spectra of **2** were similar to those of **6**, except for the A-ring moiety. The H-3 and H-5 signals in **6** were absent in **2**, whereas that for H-4 in **6** was shifted to downfield to δ 5.77 (1H, *s*) and that for H-19 at δ 0.90 in **6** downfield shifted to δ 1.27 in **2**. The ^{13}C NMR spectrum showed downfield shifts of H-3, H-4 and H-5 from δ 70.8, 37.8 and 44.6 in **6** to δ 198.6, 168.5, 124.6 in **2**, forming an α, β-unsaturated ketone moiety. The ^{13}C NMR spectrum of **2** (Table 1) was similar to that of 25*S*-spirost-4-en-3,12-dione (Domingaez, Waston, Garcia, & Martinez Davila, 1985), except for the chemical shift of the chiral centre at C-25. The chemical shift of C-25 and C-27 in the ^{13}C NMR spectrum of 25*S*-spiroketal saponins are δ 27 and 16, respectively, but those of 25*R*-spiroketal saponins are δ 30 and 17, respectively (Agrawal, Jain, Gupta, & Thakur, 1985). The relative stereochemistry of **2** was *R* as deduced from the chemical shift at δ 30.1 and 17.1 of C-25 and 27. These results suggested structure **2** for 25*R*-spirost-4-en-3,12-dione.

Table 1
^{13}C NMR spectral data of 25*R*-spiro-4-en-3, 12-dione (**2**), Hecogenin (**6**) and 25*S*-spiro-4-en-3,12-dione (**14**)

C	2	6	14*
1	35.2	36.4	35.3
2	32.3	31.0	32.9
3	198.6	70.8	198.6
4	124.6	37.8	124.7
5	168.5	44.6	168.5
6	33.5	28.2	33.7
7	31.3	31.3	31.1
8	34.2	34.3	34.4
9	54.4	55.4	54.6
10	38.6	36.0	38.7
11	37.0	37.8	37.1
12	211.9	213.6	211.9
13	54.7	55.0	54.8
14	54.7	55.7	54.8
15	31.0	31.5	31.1
16	78.9	79.1	79.1
17	53.4	53.4	53.3
18	15.8	15.9	15.9
19	16.7	11.9	16.9
20	42.1	42.1	42.7
21	13.1	13.2	13.0
22	109.2	109.2	109.7
23	31.0	31.1	25.8
24	28.6	28.7	26.0
25	30.0	30.1	27.0
26	66.8	66.8	65.2
27	17.0	17.1	16.0

* Data from (Domingaez et al., 1985).

Tribulusterine (**3**) was obtained as yellowish powder, which gave a positive reaction with Dragendroff's reagent, indicating it to be an alkaloid. The pseudomolecular formula was established as $C_{16}H_{13}N_2O_4$ by FAB mass spectrometry. The UV spectrum showed a close resemblance to that of perlolyrine (Jeffroys, 1970). Its IR spectrum showed bands at 3310, 1622 and 1529 cm^{-1}, for hydroxyl, amine and aromatic groups, respectively. The ^1H NMR spectrum exhibited a signal at δ 9.36 (1H, *br*, *s*, exchangeable with D_2O) for NH. Four mutually coupled aromatic protons at δ 8.11 (1H, *d*, $J = 8.0$ Hz), 7.60(1H, *dd*, $J = 7.4$, 1.6 Hz), 7.57(1H, *td*, $J = 7.4$, 1.6 Hz), 7.29(1H, *ddd*, $J = 8.0$, 7.4, 1.6 Hz), together with *ortho*-coupled signals at δ 8.44 and 7.86 ($J = 5.6$ Hz), indicated the presence of a 1-substituted-β-carboline nucleous in the molecule. The signals at δ 7.21 and δ 6.52 (each 1H, *d*, $J = 3.2$ Hz) were attributed to H-5′ and H-4′ of the furan ring. An aryl hydroxyl methyl proton appeared at δ 4.84. The NOESY spectrum (Fig. 1) showed a cross-peak of H-4′ (δ 6.52) with the hydroxyl methyl signal (δ 4.84) and H-5′ (δ 7.21), indicating the connection between C-1 of β-carboline and C-2′ of the furan ring. On the basis of the above results, structure **3** was assigned to tribulusterine.

The known compounds, *N*-*p*-coumaroyltyramine (Okuyma, Shibata, Kawada, Osoda, & Noguchi, 1986), terrestriamide (Ren et al., 1994), hecogenin (David, Harold, Christal, & Karen, 1990), aurantiamide acetate (Banerji & Das, 1975), xanthosine (Britmaier & Voelter, 1978), fatty acid ester, ferulic acid (Wu, Yeh, & Wu, 1995), vanillin (Wu et al., 1995), *p*-hydroxybenzoic acid (Wu et al., 1995) and β-sitosterol (Wu et al., 1995) were isolated and characterized by comparison of their spectral data with corresponding literature values.

3. Experimental

Mps: uncorr. ^1H NMR (200 and 400 MHz) and ^{13}C NMR (50 MHz) were recorded in $CDCl_3$, except where noted. Chemical shift values are given δ with TMS as int. standard. MS were recorded using a direct inlet system. UV were determined in MeOH and IR recorded in KBr.

3.1. Plant material

Tribulus terrestris was bought from a market and identified by Prof. C. S. Kouh. A voucher specimen is

deposited in the Department of Chemistry. National Cheng Kung University, Tainan, Taiwan, R.O.C.

3.2. Extraction and isolation

A MeOH extract of dried fruits (10 kg) was treated with $CHCl_3$, *n*-BuOH and H_2O, respectively. The $CHCl_3$ layer was concd to dryness and subjected to silica gel CC eluting with $CHCl_3$ containing increasing proportions of Me_2CO, to give 8 frs. Fr. 3 was rechromatographed on silica gel CC and eluted with EtOAc–*n*–hexane (1 : 9) to yield **6** (135.2 mg), **2** (76.4 mg), **7** (49.7 mg), **9** (7.2 mg) and **13** (324.6 mg), respectively. Fr. 5 was rechromatographed on silica gel CC eluted with MeOH–$CHCl_3$ (1 : 25) to afford **4** (7.8 mg), **5** (72.5 mg), **10** (5.6 mg) and **11** (3.2 mg), successively. The *n*-BuOH layer was concd and subjected to Diaion HP-20 CC, eluting with a gradient of H_2O–MeOH to afford 6 frs. Fr. 2 was rechromatographed on silica gel CC using MeOH–$CHCl_3$ (1 : 9), to yield **8** (373 mg) and unknown A (21 mg). Fr. 4 was rechromatographed on silica gel CC and eluted with MeOH–$CHCl_3$ (1 : 20), to afford **4** (27.5 mg), **5** (120.3 mg), **1** (4.3 mg), **3** (0.4 mg), **12** (3.4 mg) and unknown B (22.2 mg), successively. The H_2O layer was concd and recrystallized from MeOH to yield **8** (3.79 g).

3.3. Terrestribisamide (**1**)

Colourless powder (MeOH), mp 143 ~ 135°C. HRFABMS: Calcd. for $C_{24}H_{29}N_2O_6$, 441.2026 $[M+1]^+$, found 441.2012. UV λ_{max} nm: 319, 294, 234, 220. IR ν_{max} cm^{-1}: 3500, 2927, 1652, 1516, 1274. FABMS *m/z*: 441$[M+1]^+$, 221, 207, 193, 177, 54, 147, 138, 137, 136, 120, 107, 91, 90. ^{1}H NMR (DMSO-d_6): δ 1.45 (4H, *m*, H-2,3), 3.14 (4H, *m*, H-1,4), 3.78(6H, *s*, $2\times OCH_3$), 6.40 (2H, *d*, $J = 15.6$ Hz, H-7′, 7″), 6.76 (2H, *d*, $J = 8.0$ Hz, H-5′, 5″), 6.96 (2H, *dd*, $J = 8.0$, 1.5 Hz, H-6′, 6″), 7.09 (2H, *d*, $J = 1.5$ Hz, H-2′, 2″), 7.29 (2H, *d*, $J = 15.6$ Hz, H-8′, 8″), 7.99 (2H, *t*, $J = 5.2$ Hz, NH), 9.41 (2H, *br s*, OH).

3.4. 25*R*-*Spirost*-4-*en*-3,12-*dione* (**2**)

Colourless powder ($CHCl_3$), mp 240–241°. HREIMS: calcd. for $C_{27}H_{38}O_4$ 426.2770 $[M]^+$, found 426.2767. $[\alpha]_D$ −34.8° (*c* 0.028, $CHCl_3$). UV λ_{max} nm: 238. IR ν_{max} cm^{-1}: 2927, 1706, 1674. EIMS *m/z*: 426$[M]^+$, 398, 367, 354, 312, 269, 139, 126, 69, 55. ^{1}H NMR: δ 5.77 (1H, *s*, H-4), 4.34 (1H, *m*, H-16), 3.49 (1H, *dd*, $J = 10.8$, 2.7 Hz, H-26), 3.33 (1H, *t*, $J = 10.8$ Hz, H-26), 2.56–1.05 (22H, *m*), 1.27 (3H, *s*, H-18), 1.10 (3H, *s*, H-19), 1.06 (3H, *d*, $J = 7.0$ Hz, H-21), 0.78 (3H, *d*, $J = 6.18$ Hz, H-27). ^{13}C NMR: Table 1.

3.5. Tribulusterine (**3**)

Yellowish powder ($CHCl_3$), mp 184–185°. UV λ_{max} nm: 378, 367, 291, 284, 272, 255, 237. IR ν_{max} cm^{-1}: 3310, 2922, 2852, 1529, 1377, 1350, 736. FABMS (rel. int.) *m/z*: 265$[M+1]^+$ (10), 261 (11), 149 (11), 132 (100), 105 (42). ^{1}H NMR: δ9.36 (1H, *br s*, NH), 8.44 (1H, *d*, $J = 5.6$ Hz, H-3), 8.11(1H, *d*, $J = 8.0$ Hz, H-5), 7.86 (1H, *d*, $J = 5.6$ Hz, H-4), 7.60 (1H, *dd*, $J = 7.4$, 1.6 Hz, H-8), 7.57 (1H, *td*, $J = 7.4$, 1.6 Hz, H-7), 7.29 (1H, *ddd*, $J = 8.0$, 7.4, 1.6 Hz, H-6), 7.21 (1H, *d*, $J = 3.2$ Hz, H-5′), 6.52 (1H, *d*, $J = 3.2$ Hz, H-4′), 4.84 (1H, *s*, CH_2).

Acknowledgements

The author is grateful for financial support from the National Council of R.C.O. (NSC 87-2114-M-006-012). This work was supported in part by grants from the National Research Institute of Chinese Medicine.

References

Agrawal, P. K., Jain, D. C., Gupta, R. K., & Thakur, R. S. (1985). *Phytochemistry*, *24*, 2479.

Banerji, A., & Das, R. (1975). *Indian Journal of Chemistry*, *13*, 1234.

Bose, B. C., Saifi, A. Q., Vijayvargiya, R., & Bhatnager, J. N. (1963). *Indian Journal of Medical Sciences*, *17*, 291 [*Clinical Abstracts 59*: 1519c(1964)].

Britmaier, E., & Voelter, W. (1978). *^{13}CNMR Spectroscopy Methods and Application in Organic Chemistry*, 2nd Edn, Verlag Chemie Weinheim, New York, p. 272.

Chiang Su New Medicinal College (ed.) (1977). *Dictionary of Chinese Crude Drugs*, Shanghai Scientific Technologic Publisher, Shanghai, p. 1274.

Chiu, N. Y., & Chang, K. H. (1986). *The Illustrated Medicinal Plants of Taiwan*, *3*, P118, SMC Publishing INC. Taipei, Taiwan.

David, N. K., Harold, C. T., Christal, P., & Karen, A. W. (1990). *Journal of Chemical Society Perkin Transactions II*, 1567.

Domingaez, X. A., Waston, W. H., Garcia, S., & Martinez Davila, I. A. (1985). *Planta Medica*, *51*(6), 534.

Duan, J. A., & Zhou, R. H. (1993). *Zhongcaoyao*, *24*, 320.

Jeffroys, J. A. D. (1970). *Journal of Chemical Society* (*C*), 1091.

Jit, S., & Nag, T. N. (1985). *Indian Journal of Pharmaceutical Sciences*, *47*, 101 [*Clinical Abstracts 104*: 165379j(1986)].

Mahato, B., Shau, N. P., Pal, B. C., Chakravarti, R. N., Chakravaryi, D., & Ghosh, A. (1978). *Journal of Institution and Chemists*, *50*, 49 [*Clinical Abstracts 89*: 56460t(1978)].

Mahato, S. B., Niranjan, P., Ganguly, A. N., Miyaharo, K., & Kawasaki, T. (1981). *Journal of Chemical Society Perkin Transactions I*, 2405.

Mahato, S. B., Nianjan, P., Ganguly, A. N., Miyaharo, K., & Kawasaki, T. (1981). *Journal of Chemical Society Perkin Transactions I*, 2405.

Mathur, G. S., Nag, T. N., & Goyal, S. C. (1977). *Food Farming Agric*, *9*, 11 [*Clinical Abstracts 88*: 188472z(1977)].

Nag, T. N., Mathur, G. S., & Goyal, S. C. (1979). *Comparative Physiology and Ecology*, *4*, 157 [*Clinical Abstracts 91*: 105224h(1979)].

Okuyma, T., Shibata, S., Kawada, M., Osoda, H., & Noguchi, T. (1986). *Planta Medica*, *53*(3), 171.

Perepelitsa, E. D., & Kintya, P. K. (1975). *Khimiya Prirodnykh Soedinenii*, *11*, 260 [*Clinical Abstracts 82*: 128650t(1975)].

Ren, Y. J., Chen, H. S., Yang, G. J., & Zhu, H. (1994). *Acta Pharmaceutica Sinica*, *29*, 204.

Saleh, N. A. M., Ahmed, A. A., & Abdalla, M. F. (1982). *Phytochemistry*, *21*, 1995.

Saleh, N. A. M., & EI-Hadidi, M. N. (1982). *Biochemical Systematics and Ecology*, *5*, 121 [*Clinical Abstracts 97*: 180727v(1982)].

Sharma, H. C., Norula, J. L., & Varadarajan, R. (1977). *Chemical Era*, *13*, 261 [*Clinical Abstracts 89*: 314h(1977)].

Tomova, M. (1987). *Farmastiya*, *37*, 40 [*Clinical Abstracts 108*: 1614096d(1988)].

Tomova, M., Gyulemetova, R., Zarkova, S., Peeva, S., Pangarova, T., & Simova, M. (1981). *International Conference on Chemistry and Biotechnology of Biologically Active Natural Products*, *3*, 289 [*Clinical Abstracts 97*: 56678r(1981)].

Vasi, I. G., & Kalintha, V. P. (1982). *Comparative Physiology and Ecology*, *7*, 68 [*Clinical Abstracts 97*: 178781w(1982)].

Wu, T. S., Yeh, J. H., & Wu, P. L. (1995). *Phytochemistry*, *40*, 121.

Xie, Z. F., & Huang, X. K. (1988). *Dictionary of Traditional Chinese Medicine*, Commercial Press, Ltd, Hong Kong, p. 205.

Zafar, R., Lalwani, M., & Siddiqui, A. (1989). *Indian Drugs*, *26*, 460 [*Clinical Abstracts 111*: 121002w(1989)].