Pergamon

0031-9422(95)00248-0

Phytochemistry, Vol. 40, No. 1, pp. 121–124, 1995

0031 9422/95 $9.50 + 0.00

THE HEARTWOOD CONSTITUENTS OF *TETRADIUM GLABRIFOLIUM*

TIAN-SHUNG WU,* JYH-HER YEH and PEI-LIN WU

Department of Chemistry, National Cheng Kung University, Tainan, Taiwan 701, R.O.C.

(*Received in revised form* 12 *January* 1995)

Key Word Index— *Tetradium glabrifolium*; *Evodia meliaefolia*; Rutaceae; evomeliaefolin; evofolin-A, -B; benzo[*c*]phenanthridine alkaloid; chemotaxonomy.

Abstract—Three new compounds, evomeliaefolin, evofolin-A and -B, and 43 known compounds were isolated from the heartwood of *Tetradium glabrifolium*. Their structures were elucidated by spectral analyses. The existence of six benzo[*c*]phenanthridine alkaloids further supported that *Tetradium* is closely allied to *Zanthoxylum*, *Toddalia*, *Phellodendron* and *Fagaropsis*.

INTRODUCTION

The leaves of *Tetradium glabrifolium* (*Evodia meliaefolia*) are used as a folk medicine for the treatment of chronic ulcer of the lower extremity [1] and stomach ache [2]. Waterman *et al.* [3, 4] have reported that the presence of benzo[*c*]phenanthridine alkaloids, which are only found in genera of *Zanthoxylum*, *Toddalia*, *Phellodendron* and *Fagaropsis*, in the stem and root barks of *T. glabrifolium* supported Hartley's decision to reassign taxa from *Evodia* into the three genera: *Tetradium*, *Evodia* s.s. and *Melicope*. In order to obtain further evidence, an investigation in constituents of the heartwood of *T. glabrifolium* was undertaken. In a preliminary communication, we reported the isolation and synthesis of a benzenoid evofolin-C (**35**) from the heartwood of this plant [5]. Further examination of the heartwood of the same plant led us to isolate 54 compounds. We report now the isolation and structural elucidation of three new compounds, evomeliaefolin (**1**), evofolin-A (**2**) and -B (**3**), and 43 known compounds.

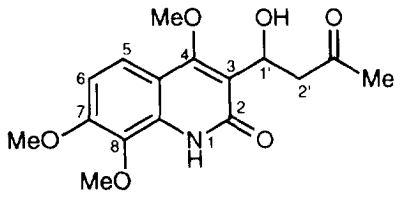
RESULTS AND DISCUSSION

The hot methanolic extract of the heartwood of *Tetradium glabrifolium* was concentrated and partitioned with chloroform and water. The water layer was then extracted with n-butanol. The chloroform and n-butanol extracts as well as insoluble portion were chromatographed on a column over silica gel or Dianion HP-20 to give evomeliaefolin (**1**), evofolin-A (**2**) and -B (**3**) and 43 known compounds.

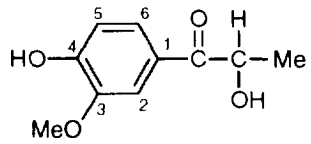
An optically active evomeliaefolin (**1**) was isolated as needles with a molecular formula of $C_{16}H_{19}NO_6$, proved by high resolution-mass spectrometry. The amide absorption at 1631 cm^{-1} in the IR spectrum and a NH signal at δ 8.94 (*br*, D_2O exchangeable) in the ^1H NMR spectrum, as well as the UV bands at 219.0, 231.4, 250.0, 257.2, 323.0, 336.4 nm, suggested the presence of a 2-quinolone skeleton [6]. In the ^1H NMR spectrum, resonances at δ 3.90 (6H, *s*, OMe-7 and OME-8) and 3.95 (3H, *s*, OMe-4) corresponded to three aromatic methoxyl groups. Two mutually coupling aromatic protons at δ 6.83 and 7.43 (each 1H, *d*, J = 9.0 Hz) which appeared further upfield than those in 4-methoxy-1-methyl-2-quinolone [7] were assigned as H-6 and H-5, respectively, followed by two methoxyl substituents (δ 3.90) attached at C-7 and C-8. A complex multiplet proton signal at δ 5.38 collapsed into double doublets (J = 8.8, 3.4 Hz, H-1′) after D_2O exchange and the diastereotopic methylene protons appeared at δ 2.67 (1H, *dd*, J = 15.4, 3.4 Hz, H-2′) and 3.30 (1H, *dd*, J = 15.4, 8.8 Hz, H-2′) which established the existence of a $-CH_2CHOH$-moiety. In addition, the presence of an acetyl group at δ 2.10 (3H, *s*) and a hydroxyl group at δ 4.83 was supported by the IR absorptions at 3400 and 1712 cm^{-1}. The EI-mass spectrum of **1** showed a $[M]^+$ ion at *m/z* 321 accompanied by the characteristic peaks at *m/z* 264 $[M - CH_2Ac]^+$ and 234 $[M - CH(OH)CH_2Ac]^+$. The above data indicated the presence of a side chain, 1′-hydroxy-3′-oxobutyl substituent in the molecule. In order to determine the positions of the side chain and the remaining methoxyl group, a NOESY experiment was conducted. It showed the subsequent NOE correlations among H-1′ and OMe-4; OMe-4 and H-5; H-5 and H-6; H-6 and OMe-7. On the basis of these data, the structure of **1** was proposed as evomeliaefolin.

Evofolin-A (**2**), a pale yellow liquid, was an optically active compound. The molecular formula was determined as $C_{10}H_{12}O_4$ by high resolution-mass spectrometry. The UV spectrum showed λ_{max} at 228.2, 279.8, 310.0 nm and the IR absorption at 1660 cm^{-1} indicated that **2** must be a conjugated benzoketone derivative. In the aromatic region of the ^1H NMR spectrum, one shielded signal at δ 6.98 (1H, *d*, J = 8.3 Hz), as well as two

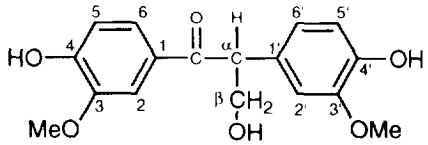
* Author to whom correspondence should be addressed.



1



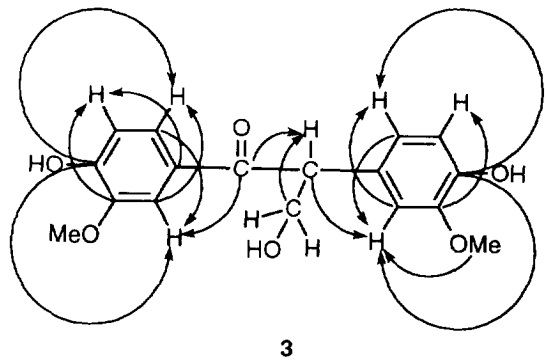
2



3

deshielded resonances at δ7.47 (1H, *dd*, J = 8.3, 1.9 Hz) and 7.54 (1H, *d*, J = 1.9 Hz), were consistent with a 3,4-disubstituted benzoketone. One aliphatic hydroxy and one aromatic hydroxy functionality in this molecule were inferred from a broad IR band at 3450 cm^{-1} and ^1H NMR signals at δ3.82 and 6.20 (each 1H, *br*) which disappeared in the presence of D_2O. Owing to the negative Gibb's test, this aromatic hydroxy substituent must be attached to C-4. The other substituent at C-3 was attributed to a methoxyl group determined from a signal at δ3.98 (3H, *s*) in the ^1H NMR spectrum. Thus a 4-hydroxy-3-methoxybenzoketone nucleus was established. The remaining part of the ketone was suggested to be a α-hydroxyethyl group because of the AX-coupling pattern at δ1.36 (3H, *d*, J = 6.9 Hz) and 5.11 (1H, *q*, J = 6.9 Hz) and the fragment ion at *m/z* 151 $[M - COCH(OH)Me]^+$. Therefore, the structure of evofolin-A was established as **2**.

The other optically active compound, evofolin-B (**3**), was isolated as pale yellow liquid with the molecular formula, $C_{17}H_{18}O_6$, determined by high resolution-mass spectrometry. Two pairs of three proton signals in the aromatic region of the ^1H NMR spectrum, which appeared at δ6.83 (1H, *d*, J = 8.3 Hz, H-5), 7.58 (1H, *d*, J = 2.0 Hz, H-2), 7.64 (1H, *dd*, J = 8.3, 2.0 Hz, H-6) and 6.72 (1H, *d*, J = 8.1 Hz, H-5'), 6.79 (1H, *dd*, J = 8.1, 2.0 Hz, H-6'), 6.98 (1H, *d*, J = 2.0 Hz, H-2'), indicated that the structure of **3** contained two 1,3,4-trisubstituted benzene rings. The former one, together with the UV absorptions at 230.6, 279.6, 306.0 nm, the IR bands at 3400, 1660 cm^{-1} and the ^1H NMR signal at δ3.86 (3H, *s*, OMe-3), was identified as a 4-hydroxy-3-methoxybenzoketone derivative by the direct comparison of the spectral data with **2**. The presence of a $-CHCH_2OH$ moiety was inferred by the ABC type signals with a methine proton at δ4.79 (1H, *dd*, J = 8.4, 5.2 Hz, H-α) together with two nonequivalent methylene protons at δ3.69 (1H, *dd*, J = 10.4, 5.2 Hz, H-β) and 4.22 (1H, *dd*, J = 10.4, 8.4 Hz, H-β) which was proved by the connectivity to the same carbon signal in the HMQC experiment. The downfield methine signal suggested the existence of two fully substituted carbon atoms, carbonyl carbon and phenyl carbon, alpha to this methine carbon. Finally, a methoxyl substituent at δ3.81 (3H, *s*) and the remaining hydroxyl group was assigned to attach at C-3' and C-4', respectively. The relationship between substituents at C-3 and C-4 and at C-3' and C-4' were confirmed by NOESY and HMBC spectra (Fig. 1). Consequently, evofolin-B was proposed to be **3**.



3

Fig. 1. ^{13}C-^1H long range correlations from the HMBC experiment for compound **3**.

The following known compounds were identified. Six benzo[*c*]phenanthridine alkaloids: bocconoline (**4**) [8], norchelerythrine (**5**) [8], oxychelerythrine (**6**) [8], 6-acetonyl dihydrochelerythrine (**7**) [9], arnottianamide (**8**) [10] and decarine (**9**) [11]; four furoquinoline alkaloids: dictamnine (**10**) [12], γ-fagarine (**11**) [13], robustine (**12**) [14] and skimmianine (**13**) [15]; two quinazolinocarboline alkaloids: rutaecarpine (**14**) [15] and hortiacine (**15**) [16]; one 2-quinolone alkaloid: 4-methoxy-1-methyl-2-quinolone (**16**) [7]; one sterol: sitosterol (**17**) [6]; one steroidal glucoside: sitosteryl glucoside (**18**) [17]; one sesquiterpenoid: atractylenolide III (**19**) [18]; one triterpenoid: lupeol (**20**) [19]; one lignan: (−)-matariesinol (**21**) [20]; one coumarin: umbelliferone (**22**) [21]; 14 benzenoids: *p*-hydroxybenzaldehyde (**23**) [22], vanillin (**24**) [23], methylvanillate (**25**) [24], methylparaben (**26**) [25], methylsyringate (**27**) [26], syringaldehyde (**28**) [27], methyl-*p*-hydroxycinnamate (**29**) [28], *trans*-4'-hydroxy-3'-methoxycinnamaldehyde (**30**) [29], 3,4,5-trimethoxybenzyl alcohol (**31**) [30], 2'-hydroxy-4'-methoxyacetophenone (**32**) [31], *p*-hydroxybenzoic acid (**33**) [32], ω-hydroxypropioguaiacone (**34**) [33], evofolin-C (**35**) [5] and hortiamide (**36**) [34]; six tetranortriterpenoids: limonin (**37**) [35], evodol (**38**) [35], 12α-hydroxyevodol (**39**) [35], 6β-acetoxy-5-epilimonin (**40**) [35], rutaevine (**41**) [35] and graucin A (**42**) [35]; four

amides: *cis*-*N*-*p*-coumaroyltyramine (**43**) [36], *trans*-*N*-*p*-coumaroyltyramine (**44**) [37], *cis*-*N*-feruloyltyramine (**45**) [38] and *trans*-*N*-feruloyltyramine (**46**) [38]; an inorganic compound: KNO_3. These compounds were isolated and characterized by the comparison of their spectroscopic data (UV, IR, NMR and mass spectrometry) with literature values. Evomeliaefolin (**1**), evofolin-A (**2**) and evofolin-B (**3**) are reported from a natural source for the first time.

The isolation of six benzo[*c*]phenanthridine alkaloids from the heartwood of *T. glabrifolium* further proved the origin of this plant is closely similar to *Zanthoxylum*, *Toddalia*, *Phellodendron* and *Fagaropsis* from the chemotaxonomical point of view.

EXPERIMENTAL

Mps: uncorr. UV: MeOH. IR: KBr unless otherwise stated. ^{1}H NMR and ^{13}C NMR: $CDCl_3$, TMS as int. standard except where noted. MS: a direct inlet system.

Plant material. Tetradium glabrifolium (Champ. ex Benth) T. Hartley (Rutaceae) used in this investigation was collected from San Dei Men, Pingtung Hsien, Taiwan in April 1990. A specimen of the plant is deposited at the herbarium of the National Cheng Kung University, Tainan, Taiwan.

Extraction and separation. The dried heartwood (15 kg) of *T. glabrifolium* was extracted with hot MeOH several times. The combined MeOH extracts were concd. under red. pres. to give a brown syrup (402 g). It was partitioned between H_2O and $CHCl_3$, and then between H_2O and n-BuOH. The $CHCl_3$ layer was dried over Na_2SO_4 and then evapd to leave a brown syrup (98 g). The n-BuOH and H_2O layers and the insoluble interface part were concd to afford n-BuOH, H_2O and insoluble portions, respectively. The $CHCl_3$ extract was directly chromatographed on silica gel eluting with $CHCl_3$–Me_2CO (9:1) to afford 9 fractions. Fr. 2 underwent CC over silica gel using n-hexane–Me_2CO (9:1) as eluent to yield **4** (46.4 mg), **5** (5.6 mg), **6** (3.3 mg), **7** (3.9 mg), **10** (25.8 mg), **11** (391.0 mg), **14** (7.5 mg), **20** (1.8 mg), **32** (5.2 mg) and **36** (2.4 mg), successively. Fr. 3 and 4 were combined and rechromatographed on silica gel eluted with C_6H_6–Me_2O (19:1) to give **8** (7.2 mg), **9** (3.8 mg), **12** (5.6 mg), **13** (3.8 mg), **15** (1.5 mg), unknown **A** (1.7 mg), **16** (5.2 mg), **19** (7.8 mg), **21** (2.0 mg), **22** (16.5 mg), **24** (6.2 mg), **28** (4.1 mg), **29** (1.5 mg), **31** (6.3 mg), **2** (1.8 mg) and **35** (0.5 mg). Similarly, the combined frs 5, 6 and 7 eluting with EtOAc–Me_2CO (40:1) gave **37** (785.2 mg), **38** (729.2 mg), **39** (699.0 mg), **40** (34.2 mg), **41** (6.0 mg), **42** (23.5 mg), **43** (1.9 mg), **44** (5.5 mg), **3** (2.1 mg), unknown **B** (154.0 mg), unknown **C** (12.4 mg), unknown **D** (2.3 mg), unknown **E** (3.2 mg) and unknown **G** (1.2 mg), successively. The solid of the insoluble portion was collected and identified as **17** (78.5 mg) after filtration. Then the filtrate was subjected to CC on Dianion HP-20 eluting with a gradient of MeOH in H_2O and rechromatrographed over silica gel to obtain **1** (1.5 mg), **18** (18.6 mg), **23** (2.0 mg), **30** (1.9 mg), **34** (5.8 mg) and unknown **F** (0.8 mg), successively. The n-BuOH extract was chromatographed on Dianion HP-20 and eluted with a gradient of H_2O and MeOH to give **33** (1.2 mg), **45** (1.5 mg), **46** (1.8 mg) and KNO_3 (35 mg).

Evomeliaefolin (**1**). Needles ($CHCl_3$–MeOH), mp 226–228°. $[\alpha]_D$-50° (MeOH; *c* 0.015). HR-MS: calcd for $C_{16}H_{19}NO_6$, *m/z* 321.1212 $[M]^+$, found 321.1213. UV λ_{max} nm (log ε): 219.0 (4.84), 231.4 (sh, 4.69), 250.0 (4.48), 257.2 (4.51), 323.0 (4.32), 336.4 (sh, 4.19) IR ν_{max} cm^{-1}: 3400, 1712, 1631, 1600, 1508. EI-MS *m/z* (rel. int.): 321 ($[M]^+$, 12) 306 (40), 278 (23), 265 (24), 264 (100), 260 (85), 234 (21). NOESY data: H-1′ (δ5.38)/H-2′ (δ2.67 and 3.30) and OMe-4 (δ3.95); H-5 (δ7.43)/OMe-4 and H-6 (δ6.83); H-6/OMe-7 (δ3.90).

Evofolin-A (**2**). Pale yellow oil. $[\alpha]_D$-28.57° ($CHCl_3$; *c* 0.021). HR-MS: calcd for $C_{10}H_{12}O_4$, *m/z* 196.0736 $[M]^+$ found 196.0734. UV λ_{max} nm (log ε): 228.2 (4.08), 279.8 (3.91), 310.0 (3.79). IR ν_{max}^{neat} cm^{-1}: 3450, 1660, 1593, 1517. EI-MS *m/z* (rel. int.): 196 ($[M]^+$, 11), 152 (20), 151 (100), 123 (11).

Evofolin-B (**3**). Pale yellow oil. $[\alpha]_D$ − 14.3° (MeOH, *c* 0.021). HRMS: calcd for $C_{17}H_{18}O_6$, *m/z* 318.1103 $[M]^+$, found 318.1105. UV λ_{max} nm (log ε): 230.6 (4.5), 279.6 (4.3), 306.0 (4.2). IR ν_{max}^{neat} cm^{-1}: 3400, 1660, 1591, 1502. EI-MS *m/z* (rel. int.): 318 ($[M]^+$, 10), 300 (16), 152 (18), 151 (100), 150 (47), 123 (12). ^{13}C NMR (Me_2CO-d_6): δ55.8 (*d*, C-α), 56.2 (*q*, 2 × OMe), 65.6 (*t*, C-β), 112.3 (*d*, C-2), 115.3 (*d*, C-5), 116.0 (*d*, C-5′), 121.9 (*d*, C-6′), 124.5 (*d*, C-6), 129.9 (*s*, C-1′), 131.3 (*s*, C-1), 146.7 (*s*, C-4′), 148.2 (*s*, C-3), 148.5 (*s*, C-3′), 152.2 (*s*, C-4), 198 (*s*, C=O). NOESY data: H-2 (δ7.58)/H-α (δ4.79) and OMe-3 (δ3.86); H-6 (δ7.64)/H-5 (δ6.83) and H-α; H-2′ (δ6.98)/H-β (δ3.69) and OMe-3′ (δ3.81).

Acknowledgement—We thank the National Science Council, R.O.C. (NSC 83-0208-M006-026) for support of this research.

REFERENCES

1. Kan, W. S. (1972) *Manual of Medicinal Plants in Taiwan*, Vol. 2, p. 373. National Research Institute of Chinese Medicine, Taipei, Taiwan.
2. *Encyclopedia of Chinese Materia Medica* (1977) Vol. 2, p. 533. Jiangsu New Medicine College, Shanghai Science and Technology Press, Shanghai.
3. But, K. M., But, P. P-H., Gray, A. I., Hartely, T. G., Kong, Y-C. and Waterman, P. G. (1987) *Biochem. Syst. Ecol.* **15**, 587.
4. Ng, K. M., Gray, A. I., Waterman, P. G., But, P. P-H. and Kong, Y. C. (1987) *J. Nat. Prod.* **50**, 1160.
5. Wu, T. S., Yeh, J. H. and Wu, P. L. (1995) *J. Chin. Chem. Soc.* **42**, 107.
6. Sangster, A. W. and Stuart, K. L. (1964) *Chem. Rev.* 102.
7. Ishii, H., Ohida, H. and Haginiwa, J. (1972) *Yakugaku Zasshi* **92**, 118.
8. Ishii, H., Ishikawa, T., Akaike, M., Tohjoh, T., Ishikawa, M. and Chen, I. S. (1984) *Yakugaku Zasshi* **104**, 1030.

9. Decaudain, N., Kunesch, K. and Poisson, J. (1974) *Phytochemistry* **13**, 505.
10. Ishii, H., Ishikawa, T., Lu, S. T. and Chen, I. S. (1984) *J. Chem. Soc., Perkin Trans. I* 1769.
11. Waterman, P. G. (1975) *Phytochemistry* **14**, 843.
12. Adinarayana, D., Ramchandraiah, P., Seligman, O. and Wagner, H. (1981) *Phytochemistry* **20**, 58.
13. Abe, F., Yahara, S., Kubo, K., Nonaka, G., Okabe, H. and Nishiok, I. (1974) *Chem. Pharm. Bull.* **22**, 2650.
14. Ishii, H., Hosoya, K. and Ishikawa, T. (1974) *Yakugaku Zasshi* **94**, 322.
15. Ishii, H., Chen, I. S., Akaike, M., Ishikawa, T. and Lu, S. T. (1982) *Yakugaku Zasshi* **102**, 182.
16. de Correa, D., Gottlieb, O. R. and de Padua, A. P. (1975) *Phytochemistry* **14**, 2059.
17. Nozakim, H., Suzuki, H., Hirayama, T., Kasai, R., Wu, R. Y. and Lee, K. H. (1986) *Phytochemistry* **25**, 479.
18. Yosioka, I., Tani, T., Hirose, M. and Kitagawa, I. (1974) *Chem. Pharm. Bull.* **22**, 1943.
19. Appleton, R. A. and Enzell, C. R. (1971) *Phytochemistry* **10**, 447.
20. Gozler, B., Arar, G., Gozler, T. and Hesse, M. (1992) *Phytochemistry* **31**, 2473.
21. Wu, T. S., Huang, S. C., Jong, T. T., Lai, J. S. and Kuoh, C. S. (1984) *Phytochemistry* **23**, 585.
22. Pouchert, C. J. (1964) *The Aldrich Library of NMR Spectra*. p. 107. Aldrich Chemical Company, New York.
23. Windholz, M. (1983) *The Merck Index* 10th Edn, p. 1419. Merck & Co., Rahway, NJ.
24. Staudo, S. J. and Bunsenges, H. B. (1968) *Phys. Chem.* **72**, 567.
25. Wu, T. S., Ou, L. F. and Teng, C. M. (1994) *Phytochemistry* **36**, 1063.
26. Shima, K., Hisada, S. and Inagaki, I. (1971) *Phytochemistry* **10**, 894.
27. Pouchert, C. J. (1984) *The Aldrich Library of NMR Spectra*, Vol. 2, p. 124. Aldrich Chemical Company, New York.
28. Koyama, H., Yoneyama, H., Fukawa, H. and Shimizu, T. (1969) *Nippon Nogei Kagaku Kaishi* **43**, 826.
29. Etse, J. T., Gray, A. I., Waterman, P. G., Ixw, G. I. and Thomas, D. W. (1988) *J. Nat. Prod.* **51**, 314.
30. Pouchert, C. J. (1993) *The Aldrich Library of* ^{13}C *and* ^{1}H *NMR Spectra*, Vol. 2, p. 367. Aldrich Chemical Company, New York.
31. Pouchert, C. J. (1993) *The Aldrich Library of* ^{13}C *and* ^{1}H *NMR Spectra*, Vol. 2, p. 856. Aldrich Chemical Company, New York.
32. Pouchert, C. J. (1983) *The Aldrich Library of NMR Spectra*, Vol. 2, p. 110. Aldrich Chemical Company, New York.
33. Achenbach, H., Stocker, M. and Constenla, M. A. (1988) *Phytochemistry* **27**, 1835.
34. Tinto, W. F., McLean, S. and Reynolds, W. F. (1992) *J. Nat. Prod.* **55**, 1676.
35. Sugimoto, T., Miyase, T., Kuroyanagi, M. and Veno, A. (1988) *Chem. Pharm. Bull.* **36**, 4453.
36. Goda, Y., Shibuya, M. and Sankawa, U. (1987) *Chem. Pharm. Bull.* **35**, 2668.
37. Okuyama, T., Shibata, S., Hoson, M., Kawada, T., Osada, H. and Noguchi, T. (1986) *Plant Med.* 171.
38. Fukuda, N., Yonemitsu, M. and Kimura, T. (1983) *Chem. Pharm. Bull.* **31**, 156.