A New Dinorxanthane and Chromone from the Root of *Tithonia diversifolia*

Yueh-Hsiung Kuo*[a,b] and Ben-Yan Lin[a]

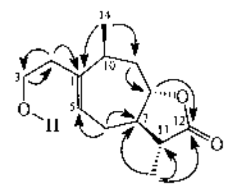
Department of Chemistry, National Taiwan University,[a] Taipei, Taiwan, R. O. C. and National Research Institute of Chinese Medicine,[b] Taipei, Taiwan, R. O. C.

Received September 18, 1998; accepted November 6, 1998

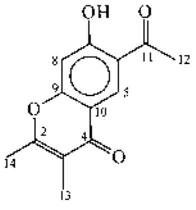
Diversifolide [4,15-dinor-3-hydroxy-1(5)-xanthen-12,8-olide], a novel dinorxanthane sesquiterpene, and a new chromone, 6-acetyl-7-hydroxy-2,3-dimethylchromone, together with four known compounds, 2-deacetyl-11β,13-dihydroxyxanthinin, 2-acetyl-2,2-dimethylchromene, 6-acetyl-7-hydroxy-2,2-dimethylchromene, and 6-acetyl-7-methoxy-2,2-dimethylchromene were isolated from the root of *Tithonia diversifolia*. Their structures were spectroscopically determined by 2D-NMR experiments, including heteronuclear multiple bond correlation and nuclear Overhauser enhancement spectroscopy.

Key words *Tithonia diversifolia*; dinorxanthane; chromone; diversifolide; 6-acetyl-7-hydroxy-2,3-dimethylchromone

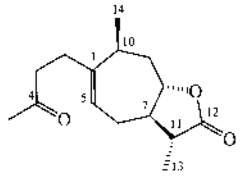
Tithonia T. diversifolia (Hemsl.) A. Gray (Compositae) is a perennial herb. Its aerial parts have been used as a traditional treatment for hepatitis and hepatoma.[1] The plant has previously been investigated and germacrane lactone, cadinane, eudesmane and chromene derivatives were isolated.[2—5] We report here that the leaf extract of this plant shows potent cytotoxicity against leukemia (HL-60, $ED_{50}=15\,\mu g/ml$) and this led us to isolate and characterize a rearranged eudesmane sesquiterpene, diversifolol, and two new sesquiterpenes, 1-acetyltagitinin and 8β-isobutyryloxycumanbranolide.[6] The methanolic extract of the roots was partitioned between *n*-BuOH and water. The *n*-BuOH layer was repeatedly purified by SiO_2 column chromatography and HPLC with an EtOAc/*n*-hexane gradient solvent system, resulting in the isolation of a new dinorxanthane sesquiterpene, diversifolide (**1**), and a new chromone, 6-acetyl-7-hydroxy-2,3-dimethylchromone (**2**) together with four known compounds, 2-deacetyl-11β,13-dihydroxyxanthinin (**3**),[7] 2-acetyl-2,2-dimethylchromene (**4**),[8] 6-acetyl-7-hydroxy-2,2-dimethylchromene (**5**),[9] and 6-acetyl-7-methoxy-2,2-dimethylchromene (**6**).[10]



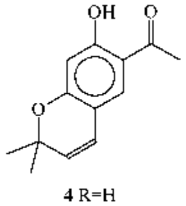
1 HMBC →



2



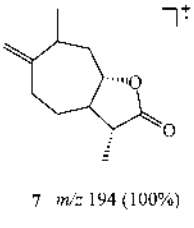
3



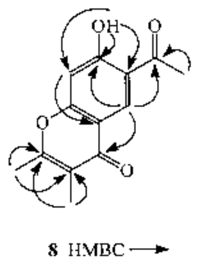
4 R=H

5 R=OH

6 R=OMe



7 m/z 194 (100%)



8 HMBC →

Table 1. NMR Data for **1** and **3** (200 and 50 MHz in $CDCl_3$)

No.	**1** δ_C	**1** δ_H	**3** δ_C
1	142.3		144.3
2	39.2	2.15—2.46 m	30.1
3	61.1	3.63 t (6.0)	42.5
4			208.0
5	121.6	5.45 br dd	119.4
6	26.3	2.15—2.46 m	26.0
7	45.0	2.15—2.46 m	44.8
8	79.1	4.65 ddd	79.0
9	35.1	1.86 ddd (α) 1.97 ddd (β)	36.0
10	35.2	2.15—2.46 m	36.2
11	39.5	2.15—2.46 m	39.0
12	179.4		179.2
13	13.9	1.19 d (6.9)	13.7
14	20.5	1.12 d (6.9)	20.4
15			29.8

$J_{5,6\alpha}=5.2$, $J_{5,6\beta}=8.4$, $J_{7\alpha,8\beta}=7.8$, $J_{8\beta,9\alpha}=3.4$, $J_{8\beta,9\beta}=11.2$, $J_{9\alpha,9\beta}=13.7$, $J_{9\alpha,10}=12.1$, $J_{9\beta,10}=6.9$.

Diversifolide (**1**), a light yellow liquid, was formulated as $C_{13}H_{20}O_3$ on the basis of high resolution electron impact mass spectrum (HR-EIMS). It contains a hydroxyl group ($3453\,cm^{-1}$), a trisubstituted double-bond (3050, 1651, and $840\,cm^{-1}$), and a γ-lactone ($1767\,cm^{-1}$). The ^1H-NMR spectrum (Table 1) revealed that **1** has two secondary methyl groups [δ 1.12 and 1.19 (each 3H, d, $J=6.9$ Hz)], a hydroxyethyl group [δ 3.63 (2H, t, $J=6.0$ Hz) and 2.15—2.46 (2H, m)] attached to an olefin group, a methine proton linked to a γ-lactone group [δ 4.65 (1H, ddd, $J=3.4$, 7.8, 11.2 Hz)], and an endocyclic trisubstituted double-bond [δ 5.45 (1H, br dd, $J=5.2$, 8.4 Hz)]. The remaining signals for methylene protons and five other protons occurred at δ 1.86 (1H, ddd, $J=3.4$, 12.1, 13.7 Hz, H_α-9), 1.97 (1H, ddd, $J=6.9$, 11.2, 13.7 Hz, H_β-9), and 2.15—2.46 (5H, m), respectively. Comparison of the ^1H- and ^{13}C-NMR data (Table 1) for com-

---

* To whom correspondence should be addressed.

pounds **1** and **3**,[7] showed that the only difference was a 2-hydroxyethyl moiety in **1** instead of a 3-oxobutyl group in **3**. Therefore, **1** was suggested to have a dinorxanthane skeleton. Using the ^{1}H–^{1}H correlation spectroscopy (COSY) and heteronuclear multiple quantum coherence (HMQC) spectral data, NMR signals were assigned. Its structure was confirmed by the heteronuclear multiple bond correlation (HMBC) (see structure **1**) technique. The base EI-MS peak of **1** at *m/z* 194 (100%) (as structure **7**) can be reasonably explained *via* a McLafferty-like rearrangement. Based on the above spectral evidence, diversifolide was established as 4,15-dinor-3-hydroxy-1(5)-xanthen-12,8-olide (only the relative configuration was elucidated), a new dinorxanthane.

Compound **2** had the molecular formula $C_{13}H_{12}O_4$ based on the exact mass [high resolution MS (HRMS)] at *m/z* 232.0732. It showed hydroxyl (3300—2700 cm^{-1}, strong hydrogen bonding), aromatic (3045, 1628, and 1598 cm^{-1}), and conjugated carbonyl (1655 cm^{-1}) IR absorption bands. The UV spectrum indicated a benzoyl group (221 and 270 nm), and the ^{1}H-NMR spectrum revealed the presence of an acetyl group [δ 2.62 (s)] attached to a phenyl residue and two singlets of a methyl group (δ 2.07 and 2.33) connected to the α- and β-positions of the chromone moiety. One phenolic proton present at δ 13.16 disappeared on addition of D_2O, indicating a hydrogen bond between a hydroxyl (C-7) and an acetyl carbonyl (C-6) as in compound **5**. Two phenyl proton singlets resonated at δ 6.62 and 8.20, and a carbon (δ_C 99.9) corresponding to the former phenyl proton was proposed to be situated between two oxygenated phenyl carbons (δ_C 169.7 and 168.8). The phenyl carbon appearing at low field (δ_C 129.0) corresponded to the latter phenyl proton (δ_H 8.20), and was considered to be located between two carbonyl groups (δ_C 204.0 and 181.9). Two ^{13}C-NMR signals at δ_C 204.0 and 181.9 were assigned as acetyl and chromone carbonyl groups, respectively. Based on the ^{1}H–^{1}H COSY and HMQC spectral data, NMR signals were assigned. Its structure was confirmed by HMBC (see structure **8**). Therefore, the structure of **2** was assigned as 6-acetyl-7-hydroxy-2,3-dimethylchromone. Although isoprenyl acetophenone-type compounds have been isolated in the *Tithonia*[8] genus, these were only of the acetylchromene-type, and this is the first time an acetylchromone-type compound has been found in this genus.

Experimental

Melting points were determined with a Yanagimoto micromelting point apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer 983G spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were run on a Bruker AM-300 spectrometer. EI-MS, UV, and optical rotations were recorded on a JEOL JMS-HX 300 mass spectrometer, a Hitachi S-3200 spectrometer, and a JASCO DIP-1000 digital polarimeter, respectively. Extracts were chromatographed on silica gel (Merck 70—230 mesh, 230—400 mesh, ASTM).

Plant Material The roots of *T. diversifolia* (Hemsl.) A. GRAY were collected in Nan-Tou, Taiwan, in 1994. The plant material was identified by Mr. Muh-Tsuen Gun, formerly a technician of the Department of Botany, National Taiwan University, and a voucher specimen has been deposited at the National Research Institute of Chinese Medicine, Taipei, Taiwan, Republic of China.

Extraction and Isolation The air-dried roots of *T. diversifolia* (13.0 kg) were extracted with MeOH (60 l) at room temperature (10 d×3). The extract was evaporated *in vacuo* to yield a residue, which was suspended in H_2O (1 l), and then partitioned twice with 1 l *n*-BuOH. The combined *n*-BuOH layers were evaporated to afford a black syrup (80 g), which was subsequently chromatographed repeatedly on silica gel using a hexane/EtOAc gradient solvent system. 6-Acetyl-7-methoxy-2,2-dimethylchromene (**4**), 6-acetyl-7-hydroxy-2,2-dimethylchromene (**5**) (41 mg), 6-acetyl-7-methoxy-2,2-dimethylchromene (**6**) (16 mg), 6-acetyl-7-hydroxy-2,3-dimethylchromone (**2**) (9 mg), 2-deacetyl-11β,13-dihydroxyxanthinin (**3**) (18 mg), and diversifolide (**1**) (5 mg) were eluted with 20%, 20%, 20%, 20%, 60%, and 60% EtOAc in the hexane solvent system, respectively. Four known compounds (**3**—**6**) were identified by comparing their physical data with that in the literature.

Diversifolide (**1**): Light yellow liquid. $[\alpha]_D^{20} = -12.5°$ (c=0.35, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3453, 3050, 1767, 1651, 840. EI-MS (70 eV) *m/z* (rel. int. %): 224 (3), 194 (100), 180 (7), 167 (14). HR-EIMS *m/z*: 224.1410 (Calcd for $C_{13}H_{20}O_3$: 224.1413). ^{1}H- and ^{13}C- NMR data are shown in Table 1.

6-Acetyl-7-hydroxy-2,3-dimethylchromone (**2**): mp 165—166°C. IR ν_{max}^{KBr} cm^{-1}: 3300—2700, 3045, 1655, 1628, 1598. UV λ_{max}^{MeOH} (log ε) nm: 221, 270. EI-MS (70 eV) *m/z* (rel. int. %): 232 (100), 217 (95), 161 (72). HR-EIMS *m/z*: 232.0732 (Calcd for $C_{13}H_{18}O_3$: 232.0736). ^{13}C-NMR ($CDCl_3$) δ: 145.3 (C-2), 132.1 (C-3), 181.9 (C-4), 129.0 (C-5), 116.1 (C-6), 169.7 (C-7), 99.9 (C-8), 168.0 (C-9), 116.3 (C-10), 204.0 (C-11), 26.6 (C-12), 20.1 (C-13), 17.3 (C-14).

Acknowledgment This research was supported by the National Science Council of the Republic of China.

References

1) Chiu N. Y., Chang K. H., "The Illustrated Medicinal Plants of Taiwan," Vol. 3, SMC Publishing Inc., Taipei, 1992, p. 254.
2) Baruah N. C., Sharma R. P., Madhusudana K. P., Thyagaraian G., *J. Org. Chem.*, **44**, 1831—1835 (1979).
3) Chowdury P. K., Baruah N. C., Sharma R. P., Thyagaraian G., *J. Org. Chem.*, **45**, 535—536 (1980).
4) Schuster A., Stockes S., Parastergiou F., Castro V., Poveda L., Jakupovic J., *Phytochemistry*, **31**, 3139—3141 (1992).
5) Bordoloi M., Baruah N. C., Ghosh A. C., *Phytochemistry*, **41**, 557—559 (1996).
6) *a*) Kuo Y. H., Chen C. H., *Chem. Pharm. Bull.*, **45**, 1223—1224 (1997); *b*) *Idem*, *J. Nat. Prod.*, **61**, 827—828 (1998).
7) Bohlmann F., Suwita A., *Phytochemistry*, **18**, 885—886 (1979).
8) Bohlmann F., Mahanta P. K., Suwita A., Antoinette A., Natu C., Zdero C., Dorner W., Ehlers D., Grenz M., *Phytochemistry*, **16**, 1973—1981 (1977).
9) Gomez F., Quinjao J. S., Perales C. A., Rios T., *Phytochemistry*, **21**, 2095—2097 (1982).
10) Bjelddane L. F., Gessmann T. A., *Phytochemistry*, **8**, 1293—1296 (1969).